biopsy. Therefore, awareness to their known expression of MDM2 and CDK4 should be made to avoid overdiagnosis.

843 USE OF BETA CATENIN IN EVALUATION OF RESECTION MARGINS OF SOFT TISSUE FIBROMATOSIS

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Background: Desmoid-type fibromatosis is a clonal fibroblastic proliferation with infiltrative growth and a tendency to recur. Since fibromatosis is known for its bland histological appearance and similarity to scar tissue and tendons, the margins' clearance represents a challenge especially in the face of recurrence. Since beta-Catenin nuclear staining is a consistent feature of this entity, we retrospectively evaluated surgical resection margins in cases of soft tissue desmoid fibromatosis using this marker.

Design: Six cases of soft tissue desmoid fibromatosis were used for this study. Follow-up time ranged from 2 to 5 years. All but two cases had negative resection margins at the time of the initial surgery. Beta-Catenin immunohistochemical stain was used to re-assess the surgical resection margins. Sections of tendons and scar tissue from unrelated cases were also stained with this marker to document the absence of beta-Catenin expression in these types of tissue.

Results: All three cases of fibromatosis with recurrence had beta-Catenin immunostaining at the surgical resection margins. The staining at the resection margins was not seen in the recurrence-free cases. In recurrent cases the staining allowed clear distinction between fibromatosis and scar tissue. Freezing of the tissue during frozen sections did not affect the staining intensity of the tissue re-submitted for permanent sections.

Conclusion: Beta-Catenin immunostaining allows for evaluation of the extent of fibromatosis remarkably well. The tissue staining was not affected by processing during frozen section. There was no staining observed in the scar or tendon tissue. Retrospective evaluation of margins using beta-Catenin showed that the staining at the inked margins was predictive of recurrence. We recommend use of this stain to rule out the recurrence of the disease as well as to achieve a complete microscopic margin clearance in cases of soft tissue desmoid fibromatosis when complete resection is feasible.

844 MYOEPITHELIAL CARCINOMA OF SOFT TISSUE. A CASE REPORT WITH CYTOGENETIC FINDINGS

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Background: Soft tissue myoepithelial carcinoma (STMC) is a very rare tumor displaying myoepithelial elements and lacking obvious ductal differentiation. Only one case report with cytogenetics is available in the English literature by which STMC seems to be a distinct entity with some resemblance to chordoma on the one hand and myoepithelioma on the other.

Design: To present the second case of STMC with cytogenetic findings.

Results: A 82-year-old female patient presented with a soft tissue tumor within the deep soft tissues in the right gluteal muscle measuring 16x13x11 cm. Histologically, the lesion was diagnosed as a myoepithelial carcinoma displaying a partly lobulated architecture with cords and nests of solid proliferations of spindled and plasmocytoid cells with frankly malignant cytomorphology. Areas of necrosis were obvious. Immunohistochemistry was partly positive for pancytokeratin , EMA, S-100 protein, and alpha smooth muscle actin, and negative for H-Caldesmon and Myf-4. MIB-1 index was 28%. Using high resolution comperative genomic hybridization a gain of 1q21-23, 9q12-q33, 16q22 and loss of 1p31-34, 1p11-22, 1q24-q44, 3p, 10q11.1-q22, 13q, 14q13-q24, 15q was detected. Further FISH analysis confirmed the 3p deletion and the monosomy of chromosomes of 13 and 15. Total DNA content measured by image cytometry proved to be diploid, DI: 0.95.

Conclusion: Our results support the hypothesis that STMC is a distinct entity, not sharing the cytogenetic alterations of salivary gland myoepithelial carcinomas and ductal carcinomas of breast with myoepithelial differentiation.

845 WILMS` TUMOR GENE (WT1) EXPRESSION IN NEUROGENIC AND MYOGENIC SARCOMAS

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Background: The Wilms` tumor gene (WT1), originally identified as a tumor suppressor gene, is overexpressed in hematologic malignancies and solid tumors. Therefore, WT1 is currently thought to play an important role in the tumorigenesis of these malignancies. It has become known that WT1 is detected in developing spinal cord, brain and skeletal muscle. The aim of the current study is to evaluate the diagnostic utility of WT1 in neurogenic and myogenic sarcomas.

Design: Formalin-fixed, paraffin-embedded sections were prepared from 131 soft tissue tumors, including 39 malignant peripheral nerve sheath tumors (MPNSTs), 10 schwannomas, 10 neurofibromas, 17 leiomyosarcomas, 10 leiomyomas, 20 rhabdomyosarcomas, 8 synovial sarcomas, 10 well-differentiated liposarcomas and 7 malignant fibrous histiocytomas (MFHs). Sections were stained with the antibody to WT1 using a biotin free detection system. The cases showing moderate to strong positivity for the antibody were regarded as positive.

Results: Thirty-two of 39 (82%) MPNSTs, 14/17 (82%) leiomyosarcomas, and 18/20 (90%) rhabdomyosarcomas were strongly positive for WT1. WT1 showed granular cytoplasmic or subplasmalemmal linear staining pattern in tumor cells. In contrast, all neurofibromas and leiomyomas were negative for WT1. Ten of 10 (100%) schwannomas were positive for WT1 although the staining intensity was relatively weak compared to those of the above described sarcomas. One of 8 (13%) synovial sarcomas, 4/10 (40%) well-differentiated liposarcomas and 2/7 (29%) MFHs were positive for WT1.

Conclusion: Within neurogenic and myogenic sarcomas, malignant tumors tended to be strongly positive for WT1, but benign tumors were negative or weak for WT1. WT1 expression in soft tissue tumors could be a useful marker for malignant neurogenic and myogenic tumors.

846 MIXED TUMOR OF SOFT TISSUE WITH PULMONARY METASTASIS

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Introduction: Mixed tumor of soft tissue is a rare disease. The prognosis is most often benign, but a minority of the tumors metastasize.

Design: We report a case of mixed tumor with pulmonary metastasis.

Results: A 58-year-old, male was admitted to the hospital because of pain in the sole of the left foot. Four months before the first admission, the patient noticed discomfort of the left sole. Thereafter, he noticed swelling and pain in the sole, and consulted a local hospital, where a tumor of the sole was surgically resected. But 1 month before the current admission, the tumor recurred and he was admitted to this hospital. The tumor located in the soft tissue of the sole, measured 3 cm in diameter, and was elastic hard, protuberant, and exposed through a skin ulcer. The surface of the tumor was red and seemed to be rich in vascularity. Radiologically, the tumor had an ill-defined border, and showed low intensity on T1- and high intensity on T2-weighted MRI. On the second and eleventh hospital days, respectively, biopsy and resection of the tumor was performed. On the 15th hospital day, the foot was amputated. Seven months after the amputation, resection of multiple bilateral pulmonary metastases was carried out. In histopathology, the tumor was composed of trabecular and ductal arrangement of epithelioid cells and solid, fibrous and myxoid areas of spindle cells. Frank anaplasia was not seen. Mitotic count was 10/10 HPF. Vascular invasion was identified. The histopathology of pulmonary metastases was almost the same as the primary tumor. Immunohistochemically the tumor was positive for vimentin, CAM5.2, AE1+AE3, EMA, S-100 protein, GFAP, and negative for desmin, alpha smooth muscle actin, CD34, CD68.

Conclusion: In this case, frank anaplasia was not seen, but mitotic count was 10/10 HPF and vascular invasion was identified. High mitotic count and vascular invasion may be a hallmark of malignancy in the mixed tumor.

Techniques

847 IMMUNOHISTOCHEMICAL EXPRESSION PROFILE OF BREAST CANCER OBTAINED BY TISSUE MICROARRY USING TWO CORES IS COMPARABLE TO LARGE-SECTION RESULTS

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Background: The immunohistochemical analysis of a large number of tumor tissues with conventional techniques is tedious and slow. It is possible by using tissue microarry technology (TMA) to sample up to 1000 tumors on one glass slide, which then can be analyzed by fluorescence in situ hybridization, RNA in situ hybridization, or immunohistochemistry. Because of the small size of the individual arrayed tissue samples (diameter 0.6 mm), the question arises as to whether these specimens are representative of their donor tumors. The aim of this study is to compare the staining result obtained by TMA with the conventional large-section technique.

Design: 80 cases of breast cancer were retrieved from the archives of our institutions to build breast cancer model. A tissue microarray consisting of duplicate 0.6 mm cores of tumor was constructed from selected one paraffin block containing tumor per case. Serial sections of donor block were then immunostained with a panel of 4 antibodies (ER, PR, Her2/neu, and p53). Twenty-six of recipient blocks, after being cored, were serial sectioned and stained for H&E, ER, PR, Her2/Neu, and p53 immunostains. H&E of recipient blocks were examined to determine how many core accurately sample the target tumor. Concordance rates were calculated for each immunostains comparing scoring result by TMA and conventional large section of recipient blocks.

Results: Target tumors were accurately sampled by two cores in 19 out of 26 recipient blocks, and only by one core in 5 blocks. Failure to sample tumor was seen in two blocks. Concordance rates of immunostain scoring result between TMA and conventional large section were 84%, 81%, 96%, and 96% for ER, PR, Her2/Neu, and p53 respectively when all 26 recipient blocks included. The rate improved to 100% for p53 immunostain and no changes for the other markers when concordance is limited to recipient blocks that have been sampled by two cores.

Conclusion: Tissue microarry is a reliable technique for examining large set of tumors. It shows immunostaining scores comparable to those obtained by conventional large-section. However, some alterations are not detected due to heterogeneity of the tumors. This shortfall can be improved by sampling target tumor by two cores and possibly by validating the technique through examining the recipient blocks H&E sections following coring procedure.

848 PRODUCTION OF HIGH QUALITY POLYCLONAL ANTIBODY OF HPV 18 E7 PROTEIN

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Background: Human Papillomaviruses (HPVs), a large family of small double stranded

DNA viruses, infect squamous epithelia of skin including anal and perianal area and mucous epithelia of larynx and genital tract. HPV infection has been implicated in the etiology of cervical cancer and more than 90% of cervical cancers contain HPV DNA. HPV 16 and 18, represent 58% and 12% in prevalence of cervical cancer, respectively. The viral DNA which integrates into the genome of cancer cells is truncated to various degrees. However, E6 and E7 open reading frame are consistently retained and expressed as mRNA or protein. Interaction with E7 protein leads to disassociation of pRb-E2F complex, and stimulates the transcription of cellular genes involved in S-phase entry.

Design: The hydrophobic region of the HPV 18 E7 protein was determined using a protein analysis software (University of Essex). Truncated region of HPV 18 E7 gene which consists of 123bp was amplified by PCR and sub-cloned into intermediate cloning vector pCR TOPO 2.1. The amplified gene was further sub-cloned into modified pET16b expression vector and the orientation was confirmed via sequencing. The recombinant plasmid was then transformed into E. coli strain BL-21 (DE3) and expressed as suggested by the manufacturer (Novagen, Inc.). Further confirmation of the targeted protein was then carried out via Western Blotting using α-Histag monoclonal antibody (SIGMA, USA). The protein was then subjected to Immuno-Metal Affinity Chromatography (IMAC) for purification. The purified protein was then extensively dialyzed. Polyclonal antiserum was produced by repeated immunization of two female New Zealand White rabbits (Animal House, USM).

Results: The HPV 18 E7 truncated protein was seen to migrate anomalously at approximately 15 KDa in combined 12.5/15% gradient SDS-PAGE. The theoretical size of normal HPV-18 E7 protein was 11.5 KDa and the respective truncated region theoretically having a size of 4.5 kDa. The anomalous migration may be due to the unusual electrophoretic behavior of the protein. The serum that has been collected from the rabbit showed positive reactivity against bacterially expressed recombinant protein as well as the human cervical

Conclusion: This study confirms the antibody produced is specific and can be used as a HPV18 E7 protein detection agent by western blotting or immunohistochemistry

849 STATISTICAL AND NEURAL PATTERN RECOGNITION METHODS FOR THE MICROSCOPIC CLASSIFICATION OF SOFT TISSUE TUMORS: A COMPARATIVE STUDY

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Background: The microscopic features of soft tissue tumors frequently overlap. Therefore, ancillary methods are frequently required for their diagnosis. In this preliminary study we compared two digital technologies of pattern recognition, for their power of classification of the following common soft tissue tumors: leiomyosarcoma, gastrointestinal stromal tumor, fibromatosis, peripheral nerve sheath tumor, dermatofibroma and dermatofibrosarcoma protuberance. The digital methods we used were: 1. Computerized morphometry: a semiautomated object (tumor nuclei) oriented method coupled with a statistical classifier (linear discriminant analysis) and 2. Automatic digital signature formation of the images coupled with two artificial intelligence neural network classifiers.

Design: Over 300 microscopic images representing 10 tumors in each diagnostic category were analyzed. Images were equally divided in training and testing sets. Computerized morphometry was done (ImageProPlus). Tumor nuclei were evaluated for size (e.g.area), shape (e.g.ellipticity) and texture (e.g.margination). Additional variables included nuclear orientation and microspatial distribution. Using statistically significant morphometrical criteria, a statistical linear discriminant function was created in order to differentiate between tumor categories. The second method involved preparation of image digital signatures (Image Finder-6, Attrasoft). Classification of signature patterns was obtained using a Boltzmann's machine based algorithm (Attrasoft) and a backpropagation neural network algorithm (Matlab).

Results: Computerized morphometry coupled with a statistical model (discriminant analysis) revealed the highest classification accuracy for differentiation between tumor categories (accuracy ranges 98% to 100% for training sets and 75-89% for testing sets). Best differentiation was obtained between malignant versus benign categories. The neural network algorithms revealed lower classification accuracies (88%-100% by back-propagation for the training sets, and 70-87% for the testing sets. Boltzmann's algorithm revealed similar results).

Conclusion: Computerized morphometry, a semiautomatic method, displayed higher accuracy rates however, being much more time consuming and observer dependent. Digital signature method coupled with artificial intelligence presented slightly lower (but still powerful) classification rates, presenting the advantage of being fully automated and non-observer dependent. Overall, both methods revealed powerful classification rates for differentiating between the soft tissue tumor categories.

Telepathology

850 TELEPATHOLOGY FOR SUPPORT IN THE DEVELOPMENT OF HEMATOPATHOLOGY IN CAMBODIA

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Background: Telepathology is a simple and cheap tool suitable for support of pathology in developing countries. Our project is focused on hematopathology. „iPath", a software conceived at the Department of Pathology, University of Basel, was used for transmission of images, clinical data and subsequent discussion of cases. The aims of this project are 1.to support diagnostic hematopathology, 2.to establish a solid basis for the future when, with increasing therapeutic possibilities the demand for more sophisticated diagnostic methods will emerge, and 3.to test the reliability of telepathology for hematopathology.

Design: Bone marrow examinations from 94 patients were performed at the Sihanouk Hospital Center of Hope (SHCH) in Phnom Phen from beginning of 2003 to March 2006. In 67% (62/94) bone marrow biopsies (BMB) and aspirates (BMA) were available, in 23% (22/94) BMB only, and in 11% (10/94) BMA only. Digital images of biopsy sections and of aspirate smears prepared locally were submitted to „iPath" including clinical data. The material was diagnosed by experts at the University of Basel.

Results: The patient's age ranged between 9 and 64 years; 36% (34/94) of the patients were <30 years. Most frequent indications for bone marrow examination were severe cytopenias, 47% (45/94). 69% (31/45) of cytopenic patients had marked reactive changes with no evidence of a hematologic disorder. 31%(14/45) patients had severely hypoplastic marrows consistent with aplastic anemia (AA). 37% (35/94) of the patients had primary hematologic disorders: acute leukemia (AL) in 10 cases, chronic myeloid leukemia (CML) in 6, chronic myeloproliferative disorders (CMPD) other than CML in 3 patients, and multiple myeloma (MM) in 1 patient. 4%(4/94) had other conditions such as primary hyperparathyroidism, malignant lymphoma and normal marrows, In 5 cases the quality of the biopsy or aspirate was not sufficient for diagnosis.

Conclusions: This study shows that bone marrow sections and smears can be diagnosed on telepathology. Diagnostic accuracy is largely dependant on the technical quality of the submitted material and on the quality of the images. Other important factors are the quality of clinical data provided and an efficient communication between the partners. The evaluation of BMB's was somewhat easier than the evaluation of BMA's, since a low power overview can be obtained for BMB's, allowing to relate the high power images to the overall impression. This is not possible on smears, making the evaluation dependant on the choice of images submitted. The diagnoses were based exclusively on morphology, since no special investigations are available at the SHCH yet. Therefore a reliable discrimination between conditions with a poorly differentiated cell population was not possible. For confirmation of the reliability of this system a quality assessment comparing the diagnoses on the original preparations with the diagnoses on telepathology is needed.

Other

851 MONOCLONAL GAMMOPATHIES: ANALYSIS OF 197 CASES IN A COMMUNITY-BASED HOSPITAL

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Background: Monoclonal gammopathy denotes the presence of Monoclonal immunoglobulin (M-protein) in the serum or urine of tested individuals. The purpose of this study is to measure the occurrence of monoclonal gammopathies in various medical conditions, including plasma cell dyscrasias.

Design: Cases with monoclonal gammopathies were retrospectively obtained from the laboratory archival system in our institution for a period of 8 months (Jan 2004-Aug 2004). Serum protein electrophoresis (SPEP) was performed in each case using the Sebia Hydrasys LC plus Hysys automated electrophoresis system. All cases with monoclonal bands detected on SPEP were confirmed and subclassified by Immunofixation (Hydragel IF, Sebia).

Results: A total of 197 cases (one test per patient), 100 females and 97 males, ages 37-96 years (mean age 69 years) were reviewed, . Four of the 197 patients (2%) had no detectable disease when tested originally or one year later. Fifty-six patients, 40 females and 16 males, (28.5%) had multiple myeloma. Of those, 18 cases had IgG 19 had IgG Kappa (including one case with associated free Lambda light chains), 1 had IgM Lambda, 4 had IgM Kappa, 1 had IgA Lambda, 10 had IgA kappa, 3 had bi-clonal gammopathies (1 case with IgG Kappa & IgG Lambda, 1 case with IgG Kappa & IgA kappa, and 1 case had IgG Kappa & IgM Kappa)}. Forty-four cases (22.4%) had a diagnosis of Monoclonal Gammopathy of Undeterminate Significance (MGUS), 10 (5.1%) had Waldenstrom macroglobulinemia, 6 (3%) had plasmacytomas. Twenty-nine cases (14.7%) had lymphomas at presentation , 8 (4%) had leukemias. Of the remaining 40 cases (20.3%), 17 (8.8%) had tumors including adenocarcinomas, astrocytomas, non-small cell carcinoma of the lung, melanoma, prostatic, ovarian, urothelial, and thyroid carcinoma, 3 (1.5%) had myelodysplastic syndromes, 4 (2%) had auto-immune hemolytic anemia, 8 (4%) had chronic renal failure and diabetes, 3 (1.5%) had Rheumatologic disorders (Paget's disease of the bone, osteoarthritis), 2 (1%) had infections, 1 (0.5%) had Polycythemia, 1 (0.5%) had G6PD Deficiency, and 1 (0.5%) case had Immune Thrombocytopenic Purpura.

Conclusion: Monoclonal Gammopathy was found to be highly associated with diseases involving the bone marrow and lympho-reticular system such as multiple myeloma, Lymphomas, Waldenstrom macroglobulinemia, and Leukemias. MGUS (22.4%) and monoclonal gammopathy associated with tumors involving other organs (20.3%) also constituted a significant percentage of the cases studied. The reason for the monoclonal expansion of a single immunoglobulin-secreting plasma cell population in what appears to be a nonmalignant manner in most cases is unknown. The proper identification of the trigger mechanism for the production of monoclonal immunoglobulin in non-hematologic diseases needs to be studied at the molecular/cellular level.

852 PERITONEAL MALIGNANT MESOTHELIOMA FIRST PRESENTING AS SISTER MARY JOSEPH'S NODULE

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