

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 protuberans. 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, Atlasoft). Classification of signature patterns was obtained using a Boltzmann's machine based algorithm (Atlasoft) 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 Penh 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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Background: Metastasis of the umbilicus or 'Sister Mary Joseph's nodule' (in recognition to the superintendent at Saint Mary's hospital, Mayo Clinic, who holds the credit for recognizing the clinical significance of this nodule) has been described as a variety of tumors types, including adenocarcinoma of primary gastrointestinal origin and other carcinomas from ovary, pancreatic, endometrium and breast. Just a few cases of umbilical metastasis from malignant peritoneal mesothelioma (MPM) have been described in the literature. This may be a consequence of the rarity of MPM, but may also reflect a failure of surgical pathologists to recognize that MPM may manifest in this way, or a failure to report such cases.

Design: To report one case of MPM at first presented as Sister Mary Joseph's nodule.

Results: A 45 year-old male presented abdominal pain for 6 months and 10k weight loss during that period. Physical exam was almost normal except for the umbilical scar that presented a blue color. The ultrasound did not show any alteration and a biopsy of the umbilical scar was performed. The biopsy was sent to another service and was inconclusive. The patient presented decline in general condition and although a possible infection was not ruled out, malignant neoplasia was the main differential diagnosis. A laparotomy was performed and the surgeon found ascitis, and multiple implants of the parietal peritoneum. The epiploon presented small nodules. The visceral peritoneum of the liver also showed implants on its surface. A biopsy of the implants and nodules was performed. Microscopic examination showed proliferation of polygonal mesothelial cells with round nuclei, conspicuous single nucleoli, and abundant eosinophilic cytoplasm. The architecture was solid, with papillary and tubular areas. The immunohistochemical study showed immunoreactivity for calretinin and CK5/6. The diagnosis of malignant mesothelioma was established.

Conclusion: We described one case of peritoneal malignant mesothelioma with umbilical metastasis presenting the Sister Mary Joseph's nodule. This is an uncommon occurrence reported in the literature.

853 MULTIPLE MONOCLONAL GAMMOPATHIES ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS IN AN ADOLESCENT FEMALE

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Background: We report a case of a 13 year old Hispanic female with a recent diagnosis of systemic lupus erythematosus (SLE) who was found to have multiple monoclonal gammopathies by immunofixation (IgA, IgM, Kappa, and Lambda). A search of the literature reveals no prior reports of this scenario.

Design: Review of needle core biopsies of kidney examined by light and electron microscopy, immunohistochemical stains, and immunofluorescence; serum protein electrophoresis and serum immunofixation gels; review of the medical chart and reference lab results.

Results: The renal biopsy was diagnosed as WHO class IV lupus nephritis. Subsequent reference lab testing was positive for anti-nuclear antibodies, anti-Smith antibodies, anti-Smith/RNP antibodies, and double-stranded DNA, supporting the diagnosis of SLE. Serum protein electrophoresis showed a total protein level below expected limits, an elevated alpha-2 fraction (21.7%), and a low gamma fraction (5.2%). Serum immunofixation revealed the presence of IgA, IgM, Kappa, and Lambda paraproteins.

Conclusion: While cases of monoclonal gammopathy in adult lupus patients have been previously described, there are no reports of multiple monoclonal gammopathies in an adolescent with SLE. We suspect that the increased number of paraproteins may be a factor in this patient's numerous morbidities; however, a definitive link between the two has not been established. We recommend that further research be undertaken to elucidate the role that multiple monoclonal gammopathies play in systemic lupus erythematosus and whether serum immunofixation may be of benefit to the clinician caring for such a patient.

854 ERDHEIM-CHESTER DISEASE: A CYTOGENETICS STUDY OF TWO CASES

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Background: Erdheim-Chester disease (ECD) is a very rare histiocytic disorder of unknown etiology that predominantly involves bone and visceral organs. Whether ECD represents a reactive or a neoplastic process has been a controversial subject since its initial description.

Design: Herein, we report for the first time cytogenetic findings of two cases of ECD diagnosed at the Mayo Clinic.

Results: The first case occurred in the right tibia of a 35-year-old male and showed the balanced chromosomal translocation t(12;15;20)(q11;q24;p13.3) among other numerical chromosomal abnormalities. The second case occurred in the retroperitoneum of a 41-year-old male and showed a normal karyotype. Both lesions expressed CD68 and were negative for CD1a and S100.

Conclusion: These findings seem to indicate that some cases of ECD are clonal histiocytic neoplastic disorders. Additional studies are warranted to confirm whether these chromosomal abnormalities are recurrent cytogenetic events.