**513 CARCINOMA EX PLEOMORPHIC ADENOMA: A RETROSPECTIVE STUDY**

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**Background:** Carcinoma ex pleomorphic adenoma (Ca-ex-PA) is a rare neoplasm of salivary glands that arises by malignant transformation of the epithelial component of pleomorphic adenoma. The variable behaviour of Ca-ex-PA is related to tumour stage, histologic grade, proportion of the carcinomatous component and extent of invasion. As an adjunct to morphology of these neoplasms, immunohistochemical assessment of various markers has been used.

**Design:** In order to describe the pathologic spectrum of these tumours and to evaluate potential prognostic factors we retrospectively studied 15 cases of Ca-ex-PA, which were found among 640 salivary gland tumours during a period of 20 years. Materials and Methods: Patients, 12 males and 3 females, ranged in age from 34-78 years (average: 61 years). H-E slides from each case were reviewed. Additionally, paraffin sections were immunostained for C-erbB-2, p53, and Ki-67.

**Results:** Twelve cases (80%) involved the parotid gland, two (13%) the submandibular gland and the remaining one the intraoral minor glands. The malignant component was invasive in 9 cases and non-invasive in 6 cases, and most often demonstrated features of adenocarcinoma (8 cases) or undifferentiated carcinoma (3 cases). Six tumours were high grade, and two of them presented with lymph node metastases. C-erbB-2 expression observed in 8 (53%) cases. Carcinomatous component of all six non-invasive tumours was positive, while benign areas were consistently negative. Positive staining for p53 was noted in 8 (53%) cases; three of them non-invasive and three high grade including the two with lymph node metastases. Twelve cases (80%) stained for Ki-67, with all six high grade tumours having high proliferative index ( nuclear staining >10%).

**Conclusion:** Ca-ex-PA is an aggressive neoplasm that accounts for 3,6% of all salivary gland tumours. In our study, Ca-ex-PA was observed in 2,4% and 7% of all salivary gland tumours and pleomorphic adenomas respectively. Grossly, Ca-ex-PA is unifocal, encapsulated mass, ranging in diameter from 1,5 to 25 cm. Histopathologic feature of the neoplasm is various combination of epithelial and mesenchymal-like components. Based on the extent of invasion beyond the capsule, Ca-ex-PA is classified as non-invasive, minimally invasive and invasive. The C-erbB-2 oncogene and its protein product are important in the pathogenesis of Ca-ex-PA. Positivity in areas with transitional features between benign and malignant components indicates that C-erbB-2 amplification may be an early step in malignant transformation. We noted the same tendency in our results. The p53 tumour suppressor gene is involved in salivary gland carcinogenesis, and its oncoprotein overexpression associates more frequently with regional and distant metastases. In our study too, the 2 cases with lymph node metastases were positive for p53. The high proliferative activity (Ki-67) correlates with both the histological grade and the metastatic potential of Ca-ex-PA, which is in concordance with our results. In conclusion, the combination of histologic features and certain immunohistochemical markers may be helpful in identifying malignant transformation and evaluating prognosis of Ca-ex-PA. However, the number of our cases is too small for definite conclusions.

**514 EVALUATION OF LYMPHATIC AND BLOOD VESSEL DENSITY IN THYROID TUMORS**

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**Background:** Thyroid tumors have distinctive metastatic pathways. Papillary carcinomas typically metastasize to regional lymph nodes through lymphatics while follicular carcinomas metastasize through blood vessels. The angiogenesis and lymphangiogenesis pattern has not been fully characterized in thyroid tumors. It is therefore of great interest to investigate whether there is difference in blood or lymphatic vessel density among various types of thyroid tumors. In this study, we used CD31 which stains both blood vessel and lymphatics, and D2-40 which stains for lymphatics to immunostain a series of thyroid tumors and compared the lymphatic and blood vessel patterns.

**Design:** Paraffin sections of 25 archived surgical specimens including 5 thyroid adenomas (FA), 6 follicular carcinomas (FC), 6 follicular variant of papillary carcinomas (FVPC) and 8 usual-type papillary carcinomas (UPC) were immunostained with mouse-anti-human D2-40 and CD31 antibodies. Five representative images of tumor, adjacent non-tumor and tumor capsule (if present) were taken from each case. Microvascular densities (MVD) were evaluated using IPlab Scientific image software and analyzed using paired t-test and ANOVA.

**Results:** All tumor samples studied have intra-tumor CD31 positive microvessels. The intra-tumor CD31 positive vessel density was higher in tumors than the adjacent non-tumor thyroid (NT) tissue. The MVD for FVPC=7.46 vs NT=1.35, p=0.038; for UPC=5.97 vs NT= 1.94, p=0.0003; for FC=6.78 vs NT=0.63, p=0.002. There is no statistically difference between adenoma (FA=4.7) and adjacent non-tumor thyroid (NT=1.71, p=0.29). The malignant tumors seem to have a higher MVD than adenomas (5.97 7.46 vs 4.7). There are no intra-tumor D2-40 positive lymphatics in FC and FA while 3 out of 14 PC have rare intra-tumor lymphatics. Adjacent non-tumor thyroid tissue as well as identifiable tumor capsule all has various amount of lymphatics. The capsule of follicular carcinoma has less D2-40 positive lymphatics compare to the adjacent non-tumor thyroid (Cap = 0.79, Non-tumor= 1.18), while it is the opposite for follicular variant of papillary carcinoma (Cap = 0.99, Non-tumor = 0.22). Comparing to follicular carcinoma, the lymphatic vessel density in tumor capsule of the follicular variant of papillary carcinoma is slightly higher (FVPC=0.99; FC=0.79).

**Conclusion:** Thyroid malignant tumors have statistically significant higher intra-tumor blood vessel density comparing to adjacent non-tumor thyroid. There are no intra-tumor D2-40 positive lymphatics present in all tumor types studied except for rare papillary carcinomas. Therefore, intra-tumor lymphatics may not play a role in the lymphatic metastasis of thyroid cancers. The lymphatics at the tumor periphery instead may prove to be important. There are slight differences in capsular lymphatic vessel density between follicular carcinoma and follicular variant of papillary carcinoma, but the significance of which is yet to be determined by additional studies.

**515 SALIVARY DUCT CARCINOMA WITH MIXED HISTOLOGICAL SPECTRUM FROM LOW-GRADE TO HIGH-GRADE. REPORT OF A CASE**

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**Background:** Salivary duct carcinoma has been well-established as a high grade malignancy due to its aggressive growth. Histologically, it resembles high grade infiltrating ductal carcinoma of the breast. Recently, low-grade salivary duct carcinoma has been described and classified as low-grade cribriform cystadenocarcinoma by the WHO 2005 edition. The association between low-grade and high-grade salivary duct carcinoma, however, has not been well-documented. We report one such case.

**Design:** Given the co-existence of mixed histological spectrum from low-grade to intermediate and high-grade carcinoma in one tissue block, we conducted immunohistochemical studies using markers including estrogen receptor (ER), progesterone receptor (PR), HER2/neu, S-100 and p53.

**Results:** The results showed uniformly negative staining for ER, PR, HER2-neu, S-100, but positive nuclear staining of tumor cells with p53 in contrast to the negative staining of benign ducts. Signal intensities of the staining vary among tumor cells of different grades. About 90% of the low-grade tumor cells showed remarkably less intense signals than intermediate and high-grade tumor cells, but the rest 10% among the low-grade tumor cells exhibited staining signal intensities comparable to the higher-grade tumor cells.

**Conclusion:** These results suggest a possible role for p53 in the tumorigenesis of salivary duct carcinoma and would support low-grade salivary duct carcinoma as a precursor of high-grade salivary duct carcinoma.

## Hematopathology

**516 HODGKIN`S LYMPHOMA IN THE COASTAL REGION OF SYRIA: MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL ANALYSIS OF 86 CASES**

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**Background:** The incidence of Hodgkin's lymphoma ( HL ) in Syria and other middle East countries is higher than in western countries and it is one of the most common lymphoma affecting the younger population . The purpose of this study was to analyze the pattern & immunophenotype of Hodgkin's lymphoma ( HL ) in the coastal region – Syria , with special reference to gender ,age and clinical stage of this lymphoma .

**Design:** We evaluated the morphological & imunohistochemical features , as well as the clinical manifestation of 86 cases of HL diagnosed in the Pathology department of Tishreen university , Lattakia, Syria in the period between 2000 – 2005 . Each case was classified according to the WHO lymphoma classification. For confirmation we used a large panel of immune stains, including CD 30 , CD 15, CD 20 , CD 3 , CD 45 , ALK and EMA .

**Results:** Most Patients presented with stage II disease. There were 22 male, 64 female. Patients range in age from 6 – 50 years with a median of 23 years. The young adult population ( 15 – 30 ) was the largest group of this study ( 50 cases , 56 % ) followed by the childhood group ( 0 – 14 ) which account (16 cases, 18.5 % ). Nodular sclerosing ( NS ) was the most prevalent subtype ( 68 cases , 79 % ) with mediastinal involvement in almost all cases , the rate of NS continued to increase during the course of study , followed by mixed cellularity MC ( 8 cases , 9 % ) . Diagnostic Reed-Sternberg cells Were positive for CD 30 in 100% , for CD 15 in 85 % and negative for CD 45 and ALK .

**Conclusion:** The results of this study revealed the different patterns of Hodgkin's lymphoma in 86 Syrian Patients & showed a high incidence of NS subtype followed by MC, this unlike studies from other Middle East countries where the MC was the most common subtype . The age distribution was similar to other developing counties with an earlier presentation than US and Western countries.

**517 MARGINAL ZONE B-CELL LYMPHOMA IN JAPAN: LOW FREQUENCY OF NODAL DISEASE**

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**Background:** Frequency of marginal zone B-cell lymphoma(MZBCL) was estimated among lymphoproliferative diseases diagnozed and treated in Osaka, Japan from 1999-2004.

**Design:** The total number of registered cases was 1883, among which 1579 pateints were diagnosed as having malignant lymphomas. These were 1431(90.6%) of non-Hodgkin`s lymphoma(NHL) and 148(9.4%) of Hodgkin`s lymphoma. Among cases of NHL, 1092(69.2%) were B-cell lymphomas and 297(18.8%) were T/NK-cell lymphomas.

**Results:** 101(7.1%) of 1431 NHL cases were MZBCL: 95 cases(6.7%) of mucosa-associated lymphoid tissue(MALT) type, 4(0.3%) of splenic, and 2(0.1%) of nodal MZBCL. Age of patients at presentation and sex ratio in our series were rather similar to those in western countries. Three of four patients with splenic MZBCL showed a leukemic blood picture at presentation. One of the pateints with splenic MZBCL had been suffering from primary biliary cirrhosis.

**Conclusion:**Through review of pertinent literature, it is concluded that nodal MZBCL like chronic lymphocytic leukemia is much less frequent in Japan than in western countries.

**518 ACCELERATION OF FUNCTIONAL AND HISTOLOGICAL RECOVERY OF TRANSECTED SPINAL CORD IN MICE AFTER TRANSPLANTATION OF BONE MARROW STEM CELLS (BMCS) OR ADMINISTRATION OF G-CSF**

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**Background:** Recovery of central nervous system injuries is hindered by limited neurogenesis, inability of remyelination and re-established functional neural connections. Recent studies demonstrated, that adult BMCs transdifferantiate into various non-hematopoietic cell lineages and/or participate in injure healing by promoting endogenous recovery mechanisms. The present study aimed at clarifying whether BMCs or G-CSF-mobilized BM cells could home to the injured spinal cord and promote tissue repair.

**Design:** Fifty-four C57Bl/6 female mice, 10-12weeks old, underwent complete spinal cord transection (SCT) at T10-T11 vertebras and separated in 3 groups. Group A received G-CSF 200ig/kg/day for 7 days and group B received 106 mononuclear BMCs iv from male C57Bl/6 donors. Group C was the control group. Animals were sacrificed at 48 hours, 1, 3 and 5 weeks postoperatively. All animals were functionally assessed by Khun-Wrathal tests and videotaped. Histologically, lesion length and scar formation were measured in ìm rostrocaudally in Luxol Fast Blue and Van Gieson histostains. Immunohistochemistry was performed for S-100, GFAP, Synaptophysin, CD34 and double immunostains for Ki-67. Mann-Whitney U test was used for statistical analysis.

**Results:** After 48 hours all mice were paralyzed in hind limbs. In 3rd and 5th week there was significant improvement in hind limb reflex and coordinated motor function mainly in Group A ($p< 0,05$) and less in Group B compared to control Group. Histologically, spinal cord damage consisted of primary lesion (PL) and dorsal column lesion (DCL). PL in all mice characterized by neuronal cell loss, hemorrhagic infiltration and debris, 48 hours after SCT. In control Group until 3rd week there was a gradual development of fibrous tissue which divided the spinal cord in two entirely separated parts and began to regress after 5 weeks. PL was maximal in size in first week in Group A and B mice and rapidly regressed after 3rd week showing high revascularization. DCL in all animals consisted of white matter dysmyelination and cavitation, which extended rostrocaudally and reached maximum size in 3rd week. Thereafter it declined significantly in control Group. Group A and B mice showed a slight acceleration of DCL recovery. More proliferative oligodendrocytes and astrocytes were found in Group A and B than in Group C.

**Conclusion:** Transplantation of BMCs or G-CSF-mobilized BM cells accelerate functional and histological recovery after SCT in mice probably participating in wound healing process of primary lesion. They may also initiate endogenous tissue repair through a paracrine effect stimulating the proliferation of neural "supportive" cells such as, oligodendrocytes and astrocytes. Their transdifferantiation into neural cells is under investigation. G-CSF administration in a mobilization scheme or BMCs might offer a novel therapeutic approach for the treatment of severe spinal cord injuries in humans

**519 EXPERIMENTAL ARTHRITIS IN RATS RESEMBLING HUMAN RHEUMATOID ARTHRITIS. FUNCTIONAL AND HISTOLOGICAL AMELIORATION OF ARTHRITIS FOLLOWING INFUSIONS OF BONE MARROW CELLS IN UNCONDITIONED HOSTS**

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**Background:** Autologous bone marrow transplantation following immunoablative conditioning ameliorates autoimmune diseases by regenerating an 'antigen naive' immune system. On the other hand, it has been recently shown the potential of adult bone marrow stem cells (BMCs) to migrate into injured tissues and convert into the endogenous cell type. We investigated the in vivo effect of BMCs in experimental arthritis (EA) of unconditioned hosts and their in vitro effect in cultured arthritic fibroblast-like synoviocytes (FLS).

**Design:** EA was induced in female Fisher rats by subcutaneous injection of Freund's Complete Adjuvant (FCA) in the tail base. BMCs from male donors were infused in the retroorbital vein of unconditioned hosts, either before (2, 5 and 7 days post FCA) or after the onset of arthritis (23 days post FCA). The severity of arthritis was evaluated by a clinical and histological scoring system. The influence of secreted, by the BMCs, soluble factors in the apoptosis and proliferation rate of arthritic fibroblast-like synoviocytes (FLS) was also studied in a transwell system.

**Results:** In all groups foot joints were the most severely affected sites. Rats were clinically arthritic at 12-14 days after the induction of arthritis. Arthritis severity index was greater in the control group, with erythematous, swollen and stiff joints and histologically extensive erosive lesions in the cartilage and bone without however the presence of typical rheumatoid nodules. In contrast, rats treated with BMCs had significantly lower arthritic scores (more than 2-fold) and preserved a rather normal architecture of the joint with mild focal synovial hyperplasia or panus formation and reduced abnormal chondroplasia or osseous damage as compared to control rats. The histological differences between the two groups were obvious from the first day of arthritis through the day of sacrifice (+21 days post EA induction). Proliferative synoviocytes demonstrated as ki-67+4-propyl-hydroxylase positive cells by immunohistochemistry, were less in transplanted rats. Engraftment of donor-BMCs was detected in synovial membrane by immunohistochemistry for the sry protein but not in the spleens by PCR for the sry gene. FLS showed increased apoptotic death and reduced regenerative capacity when co-cultured with BMCs.

**Conclusion:** Infusion of BMCs, especially before the onset of EA, significantly reduced the severity of arthritis probably by homing of BMCs into the target joints where they may exert an immunomodulatory effect expressed as increased apoptosis and reduced proliferation of synoviocytes. Whether transdiferentiation events are also participating in this process or whether subpopulations of cells inside the bone marrow (i.e mesenchymal stem cells) may also contribute in this beneficial effect is currently under investigation.

**520 FREQUENCY AND DISTRIBUTION OF FOXP3+ REGULATORY T-CELL INFILTRATION IN FOLLICULAR LYMPHOMAS IS UNRELATED TO DISEASE GRADE OR UNDERLYING CYTOGENETIC ABNORMALITIES**

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**Background:** Regulatory T-cells (Tregs) with the phenotype CD4+, CD25+, and FOXP3+ can home to germinal centers (GC) after T-cell activation where they can suppress GC T-helper (Th) cells and Th cell stimulated B-cell responses and antibody secretion. It has also been shown that GC B-cells can recruit Tregs. Thus, we decided to investigate whether follicular lymphomas (FL), the neoplastic counterpart of GC B-cells, retain the normal pattern of infiltration and frequency of Tregs and whether differences between tumors could be attributed to a difference in their underlying cytogenetic abnormalities, grade, T-cell subset ratios, or immunoglobulin (Ig) isotypes.

**Design:** Immunohistochemical (IHC) staining was performed with an anti-FOXP3 antibody (mouse monoclonal, ebioscience, San Diego, Ca) on 28 well-characterized FL and 4 control tissues (2 inflamed tonsils, 2 reactive lymph nodes). A semiquantitative grading system (0-3+) was used to score the distribution of FOXP3+ T-cells within the neoplastic follicles as well as in the interfollicular/perifollicular (IF/PF) areas compared to non-neoplastic controls. The scores were correlated with the grade, presence or absence of IgH/BCL2 or BCL6 translocations by G-banded karyotyping and FISH analysis, and CD4: CD8 ratios and Ig isotype by flow cytometry. Statistical analysis was performed using linear correlation and Fisher's exact test.

**Results:** The 28 FL included 8 FL1, 13 FL2, 6 FL3a, and 1 FL3b. Sixteen FL had a translocation t(14;18), 3 had BCL6 translocations, 3 had both, and 6 lacked both translocations. Data regarding Ig isotypes and CD4:CD8 ratios was not available for 10 and 3 cases, respectively. CD4+ T-cells predominated in all cases and CD4:CD8 ratios ranged from 1.7:1 to 10:1. Nine (32%) FL had a similar frequency and distribution profile of FOXP3+ cells as controls, 6 (22%) had isolated increases in follicular FOXP3+ cells, and 9 (32%) had isolated increases in IF/PF FOXP3+ cells. Of the 4 (14%) cases with increased FOXP3+ cells in both locations, an IF/PF predominant pattern was seen in 3 cases and follicular predominance in 1 case. The distribution pattern or frequency of FOXP3+ cells did not correlate with FL grade, type of translocation, CD4:CD8 ratios, or Ig isotype.

**Conclusion:** FOXP3+ Tregs are increased in a high proportion of FL, perhaps responsible for host immune evasion or suppression. Tregs show marked heterogeneity in their distribution and frequency, both of which do not correlate with a few known prognostic factors. Further studies are required to determine mechanisms that regulate Treg homing in FL and the impact of Tregs on the biologic course and response to chemotherapy in FL.

**521 EBV-ASSOCIATED DIFFUSE LARGE B-CELL LYMPHOMA IN THE IMMUNOCOMPETENT: A CLINICOPATHOLOGICAL STUDY**

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**Background:** EBV is one of the most common infectious agents of humans and has been implicated in Burkitt lymphoma, other B-cell lymphomas in immunosupressed patients, T-cell, and T/NK cell lymphomas and Hodgkin lymphoma in immunosupressed and immunocompetent patients. EBV associated diffuse large B-cell lymphomas in immunocompetent persons have been reported sporadically, but have not been well described. We describe a series of three apparently immunocompetent patients who had unusual clinical presentations of an EBV positive diffuse large B-cell lymphoma.

**Design:** Biopsy specimens from three patients with EBV-associated diffuse large B-cell lymphomas were identified in the files of the Hematopathology Laboratory, Department of Pathology at our institution between 2003-2005. Cases were selected based on the presence of confirmed Epstein Barr Virus infection and no clinical evidence of immunocompromise (negative HIV test, and normal white blood cell count with CD4/CD8 ratios). Clinical histories were retrieved through chart review. A panel of immunohistochemical markers (CD2, CD3, CD4, CD 5, CD7, CD15, CD19, CD30, CD56, CD79a, CD138, CD68, CD20, CD45, CD8, TIA-1, Granyzme B, bcl-2, and bcl-6) was performed. Immunophenotypic analysis by flow cytometry was also performed in two cases. A panel of monoclonal antibodies was used, including CD19, CD20, CD22, immunoglobulin kappa and lambda light chains, CD10, CD3, CD4, CD5, CD8, CD23, CD25, sIgM, sIgD, sIgG, and sIgA. In situ hybridization studies for EBV small nuclear RNAs (EBER) were performed in all cases. Molecular analysis for immunoglobulin heavy and light chain and T-cell receptor gamma chain rearrangements using a mixture of consensus V and J primers and a polymerase chain reaction method was also performed.

**Results:** There were two male and one female patients, aged 23-78. Each patient had an unusual clinical presentation and no evidence of immunodeficiency. The first presented with fulminant liver failure leading to an emergency liver transplantation, the second patient was being treated with interferon for Hepatitis C infection when he developed the lymphoma and the third presented with massive splenomegaly and progressive

pancytopenia developing over a two-year period and had a rapid downhill clinical course after splenectomy. All patients had splenomegaly at presentation. Two cases showed positive immunolabeling of tumor cells for B-cell markers (CD19, CD20 and CD79a) by immunohistochemistry and by flow cytometry. In one case, the B-cell nature of the neoplasm was betrayed only through molecular studies. All three cases were proven to have clonal B cell populations by PCR studies. In all the cases, the tumor cells co-expressed EBV associated LMP1 antigen, EBV-RNAs (EBER) and patchy to diffuse CD30, and showed an aggressive clinical presentation. Two patients had a fulminant clinical course that led to their demise.

**Conclusion:** Our findings suggest that EBV-associated diffuse large B cell lymphomas may occur in apparently immunocompetent. These lymphomas usually take on an aggressive clinical course and that their clinical presentation may be unusual. These findings need to be investigated further in larger study groups.

### 522 POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER, POLYMORPHOUS B-CELL TYPE WITH UNIQUE CYTOGENETIC ABNORMALITIES: A WORSE PROGNOSIS?

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**Background:** Posttransplant lymphoproliferative disorder (PTLD) is a relatively rare, albeit well-known, consequence of immunosuppression in solid organ or bone marrow allograft recipients. PTLDs comprise a spectrum of lymphoid proliferation ranging from early Epstein-Barr Virus driven polyclonal proliferations resembling infectious mononucleosis to either B-cell or T-cell lymphomas. Classification of PTLDs routinely employs morphologic and cytologic examination, immunophenotyping via flow cytometry and immunohistochemisty, and karyotype and molecular analyses to determine gene rearrangement. In the literature, there are a few reported cases of clinically aggressive polymorphic PTLDs lacking monoclonality by flow cytometry and gene rearrangement analyses, but which by cytogenetic analysis reveal a malignant clone. We present a case of Posttransplant Lymphoproliferative Disorder, polymorphous B-cell type, with no evidence of monoclonality by flow cytometry, and with immunoglobulin heavy chain gene rearrangement, with a malignant clone by cytogenetic analysis. Furthermore, the cytogenetic abnormalities identified have heretofore not been reported in PTLDs.

**Results:** At the time of presentation, the patient was a 3 year-old boy born with Shone Syndrome (multi-level left ventricular outflow tract obstruction, severe subaortic stenosis, aortic valve stenosis, and aortic coarctation). After one initial operation, his coarctation and subaortic stenosis remained refractory to surgical intervention, necessitating a Ross conal operation. Subsequent to this procedure, the patient developed ventricular tachycardia, severe hypotension, and cardiac arrest, requiring extracorporeal membrane oxygenation (ECMO). Attempts at weaning the patient from ECMO were unsuccessful; the only viable option remaining was cardiac transplantation, which was successfully performed. Eighteen months status-post heart transplant, the patient presented to our hospital with an approximate one month history of weight loss, diminished appetite, somnolence, and fever. Chest radiographs revealed hazy multifocal infiltrates suspicious for infection. The patient underwent videoassisted thoracotomy and wedge biopsy for further diagnosis. Histologic examination revealed a polymorphous posttransplant lymphoproliferative disorder of B-cell type as determined by flow cytometry and immunohistochemistry, without evidence of monoclonality. Molecular genetic testing revealed immunoglobulin heavy chain gene rearrangement. Cytogenetic analysis revealed an abnormal male karyotype (48 XY, +9, +12).

**Conclusion:** Cytogenetic abnormalities previously reported in PTLDs have not included either trisomy 9 or trisomy 12, which are associated with adenocarcinomas and pituitary adenomas and B-cell chronic lymphocytic lymphoma, respectively. Of particular interest is the association trisomy 12 has with lymphoma. Does the presence of this malignant clone portend a bad prognosis for this patient? Three years status-post diagnosis, after responding initially to reduction in immunosuppression but requiring chemotherapy for ultimate disease response, the patient remains symptom and tumor free. It is likely that the identification of malignant clones in PTLDs via cytogenetic analysis renders little, if any, significance to patient outcome.

### 523 MORPHOLOGIC AND IMMUNOHISTOCHEMISTRY CARACTERIZATION OF HUMAN MESENCHYMAL STEM CELLS DIFFERENTIATED INTO OSTEOCYTES, ADYPOCYTES AND CHONDROCYTES FROM DISTINCT SOURCES

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**Background:** Human Mesenchymal stem cells (hMSCs) are pluripotent adult cells with high self-renewal capacity. Besides their wide therapeutic potential, it has been postulate that uncommitted hMSCs may evolve spontaneous genetic changes leading tumorigenicity. These cells can be usually isolated and expanded in culture from bone marrow (BM) and human umbilical cord blood. Herein, we reported an assay that undifferentiated hMSCs were collected from other sources, expanded in culture and differentiated into chondrocytes, adipocytes and osteocytes to be submitted to morphological, phenotipic and molecular comparison.

**Design:** hMSCs isolated from umbilical vein and artery, saphenous vein and BM were expanded and differentiated in vitro by distinct milieus: α Mem SBF supplemented or not with insulin, dexamethasone, indometacin, ascorbic acid, β-phosphoglycerate, DMEM without SBF plus TGFβ3, Na+ piruvate, dexamethasone, ascorbic acid and insulin-transferrin-selenium). These cells were further characterized by different hystochemical reactions, immunohistochemistry for anti-collagen II and RT-PCR for osteopontin and PPARγ2 expression.

**Results:** hMSCs were easily collected and expanded regardless the source. Despite of undifferentiated hMSCs from different origins showed similar morphologic (fibroblastoid features with central nucleus and conspicuous nucleolus) and phenotypic features (CD73+, CD105+, CD90+, CD29+, CD54+, CD44+, CD34+, CD45+, CD14+, CD51/61+, KDR+), the morphology and genetic expression of respective differentiated cells were distinct.

**Conclusion:** This experimental model suggests that hMSCs can be colleted from other sources than BM, providing a useful alternative method to obtain these cells.

### 524 DIFFUSE LARGE B-CELL LYMPHOMA IN A PATIENT WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA: A CASE REPORT

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**Background:** Large granular lymphocyte (LGL) leukemia is associated with B cell immune dysregulation resulting in a variety of autoimmune disorders and serological abnormalities. It is also associated with the development of B cell malignancies. Conversely, monoclonal and polyclonal LGL T cell proliferations have been identified among patients with pre-existing B cell malignancies. The relationship between the two major branches of the acquired immune system in the setting of lymphoproliferative disorders remains unclear. Observations of LGL leukemia may provide insight into the discussion.

**Design:** We report a case of a patient with treated LGL leukemia who subsequently developed diffuse large B cell lymphoma (DLBCL).

**Results:** A 77 year old female who presented in 1999 with complaints of progressive fatigue and occasional night sweats. Physical exam was unremarkable, and she had no complaints suggestive of rheumatoid arthritis. Her complete blood count revealed an absolute lymphocytosis (5850 k/cmm), anemia (Hb 8.7 and Hct 26.6) and mild thrombocytopenia (132,000). Peripheral blood smear review found that a majority of the lymphocytes had abundant cytoplasm with small azurophilic granules. Flow cytometry identified a population of lymphoid cells expressing T-Cell markers (CD2+, CD3+, CD5+, CD7+dim). These cells predominantly expressed CD8 (CD4+:CD8+ ratio = 1:4.5). Dim expression of CD16/56 was noted on the population of CD3+ T cells. T cell receptor gene rearrangement was not performed. Treatment with Cytoxan and prednisone was initiated. Methotrexate and cyclosporin were added to the regimen with limited improvement in LGL lymphocytosis and anemia. She was given two courses of 2-CDA in May 2000 and Jan 2001. Absolute lymphocyte counts were consistently below 5,000/il from May 2000 onward. On September 16, 2003 she was reported to have coughed a large amount of blood and became unresponsive and was found asystolic by emergency medical personnel. Autopsy revealed extensive mediastinal and retroperitoneal adenopathy. A 3.8 by 3.2 by 2.8 cm peribronchial mass was identified in the right lung hilar region. The mass had focally eroded the wall of an adjacent pulmonary artery. An adjacent 1.5 cm bronchovascular fistula explained her massive hemoptysis. Histologic review of the mass found a diffuse sheet of lymphoid cells predominantly composed of medium to large sized cells with irregular nuclear contours and a single, centrally-located nucleolus. This population of cells showed positive cytoplasmic staining for CD20.

**Conclusion:** An increased incidence of DLBCL, immunoblastic and centroblastic types, has been observed in a variety of primary immunodeficiency states. At the time of writing, we are unaware of any previously reported cases of LGL leukemia preceding diffuse large B cell lymphoma. Multiple authors have reported cases of B-cell leukemia and lymphoma arising in the setting of LGL leukemia. Monoclonal and polyclonal expansion of LGLs have been observed in the presence of hematologic malignancies suggesting an anti-tumor role for the LGL. Though a direct link of LGL leukemia to B cell malignancies remains highly speculative, our case of DLBCL following LGL leukemia adds to a growing number of reports of suggesting a relationship between the two.

### 525 RAPIDLY FATAL EPSTEIN-BARR VIRUS-ASSOCIATED ANGIOCENTRIC AND ANGIODESTRUCTIVE LYMPHOPROLIFERATIVE DISORDER

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**Background:** Epstein-Barr virus-related angiocentric and angiodestructive lymphoproliferative disorders, historically sometimes called `angiocentric immunoproliferative lesions`, generally fall under one of two categories: “lymphomatoid granulomatosis (LYG)” and “extranodal NK/T-cell lymphoma, nasal type”.

**Design:** Fifty-nine year old man presented with a three-week history of nausea, diarrhea and right upper quadrant pain. CT scan showed a right suprarenal mass, suspicious for malignancy. Further investigations by MRI raised the possibility of liver metastases; some intrathoracic lymphadenopathy and right pleural effusion were noted. While attempts were made to obtain a tissue diagnosis, the patient’s condition steadily deteriorated as he developed shortness of breath, anasarca, and fever of unclear etiology. Laboratory tests showed mild leukopenia and anemia, hypoproteinemia, and slightly elevated serum LDH. He was HIV negative. Given the rapidly deteriorating clinical status and the suspicion of widely disseminated malignant disease, resuscitation attempts were abandoned. Permission was granted for a full and unrestricted autopsy.

**Results:** At autopsy, the macroscopic impression was one of malignant disease, with multi-organ involvement, including lungs, liver, right adrenal bed (massively), paratracheal and para-aortic lymph nodes, spleen and skin. Histological examination revealed extensive effacement of tissue architecture and necrosis by an angiocentric and angiodestructive lymphoproliferative process. The atypical infiltrates showed a broad morphologic spectrum, including predominantly small to medium-sized mature-appearing lymphocytes admixed with numerous large markedly pleomorphic cells, exhibiting multilobulated /

(vaguely) Reed-Sternberg cell - like to anaplastic nuclei, with nuclear viral inclusion-like bodies. The bone marrow showed features of hemophagocytosis. Immunohistochemical studies, performed on paraffin-embedded tissue, showed the large atypical cells to be variably positive for CD 30 and CD 15; and, negative for CD 20, CD 56, CD 57, CD 8, CD 20, CD 79a, ALK-1, CD 34, and pancytokeratin marker AE1/AE3. CD 3 showed focal weak staining. CD 4 stain was equivocal. Double-staining studies showed a number of dividing (Ki 67+) cells to also be (weakly) CD 3-positive and CD 56-negative. In-situ hybridization for Epstein-Barr virus-encoded small nuclear RNAs (EBER) was extensively positive, including most of the large atypical cells. The infiltrate stained negative for CMV and HSV. Molecular genetics studies, performed on snap-frozen liver, were positive for clonal integration of the EBV genome; and, negative for immunoglobulin heavy chain (IgH), and T-cell receptor (gamma & beta chain) gene rearrangements.

**Conclusion:** The morphologic, immunophenotypic and molecular genetics findings are consistent with involvement by an "angiocentric immunoproliferative lesion", with unusual features. With respect to subclassification (according to the WHO 2001 classification), we favor the possibility of `extranodal NK/T-cell lymphoma, nasal type` over LYG, based on the CD 3-positivity (albeit weak); and, the paucity of evidence for a B-cell immunophenotype in the large atypical EBV+ cells, absent detectable IgH gene rearrangement.

### 526 SURVIVIN STAINING OF BONE MARROW BIOPSIES DISTINGUISHES INHERITED FROM ACQUIRED CYTOPENIAS: A STUDY OF 212 PATIENTS

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**Background:** Cell cycle regulation plays a major role in controlling hematopoiesis. Loss of appropriate mechanisms regulating cell cycle may lead to increased bone marrow (BM) apoptosis with subsequent cytopenias or to the accumulation of DNA-damaged and potentially harmful cells. A dysfunctional apoptotic pathway may play a role in increasing the risk of leukemogenesis, a feared complication for patients with inherited bone marrow failure syndromes (IBMFS). In a previous study, our group reported increased expression of p53 protein with no correlative increase in apoptosis as identified by caspase-3 expression in BM from patients with IBMFS. These results prompted our group to study the expression of survivin, a member of the inhibitors of apoptosis proteins (IAP), to further the understanding of cell cycle abnormalities in BM diseases.

**Design:** We studied BM biopsies of 212 patients immunohistochemically using a rabbit polyclonal antibody to survivin (ab17392, Abcam Inc., Cambridge, MA). The patients had the following diagnoses: IBMFS 57 (Shwachman-Diamond Syndrome 17, Fanconi Anemia 16, Diamond-Blackfan Anemia 15, dyskeratosis congenita 8, severe congenital neutropenia 1), unclassified inherited cytopenia (UIC) 19, acquired aplastic anemia (AA) 13, low-blast refractory cytopenia (RC) 32, and acquired cytopenias of various nutritional, medication-related, and organ failure etiologies (AC) 91. In addition, 19 hematologically-normal control subjects were included.

**Results:** The percentage of cells staining for survivin was significantly higher in IBMFS and UIC compared to other groups. The mean percentage of survivin-positive BM cells was as follows: IBMFS 64%, UIC 42%, AA 6%, MDS 7%, AC 10%, and control marrows 7% ($p<0.0001$ and $p<0.002$ when IBMFS and UIC are compared to other groups respectively). There was no statistical difference among the groups within the IBMFS category. The percentage of cases with intense expression of survivin (>40% of BM cells) was as follows: IBMFS 72%, UIC 37%, MDS 0%, AA 0%, AC 1%, and control marrows 0% ($p<0.001$ and $p<0.03$ when IBMFS and UIC are compared to other groups respectively). IBMFS differed from UIC in the percentage of survivin positive cells ($p=0.02$) and the percentage of cases with intense survivin expression ($p=0.01$).

**Conclusion:** BM survivin expression may be used as a tool to help differentiate inherited from acquired cytopenias. A high percentage of BM survivin-positive cells (particularly >40%) is characteristic of inherited bone marrow disease. Since we previously found p53 protein to be expressed at high levels in IBMFS, we suggest that survivin may play a major role in counteracting the pro-apoptotic signals of p53 and in limiting the activity of the apoptotic pathway. The balance between p53 and survivin may shed light on the cellular mechanisms of development of myelodysplastic syndromes and acute leukemia in IBMFS.

### 527 PLASMACYTIC-TYPE POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER PRESENTING IN BONE MARROW

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**Background:** Posttransplant lymphoproliferative disorder (PTLD) is an entity which is seen in patients following solid organ or bone marrow allograft. The incidence of PTLD among marrow allografts is rare, occurring in less than 1% of cases. Most are observed within one year posttransplant and present in lymph nodes or as solid tumors, without bone marrow involvement. The majority of cases occurring within one year posttransplant are associated with Epstein-Barr virus (EBV) infection. Conversely, most cases arising after one year posttransplant are EBV negative. We encountered an unusual case of EBV-positive, plasmacytic-type PTLD arising in the bone marrow of a patient seven-and-one-half years after she underwent marrow allograft for treatment of acute myelogenous leukemia (AML).

**Case:** A 50-year-old woman was diagnosed eight years prior with AML with multiple chromosomal abnormalities (including MLL) and was treated with chemotherapy, followed by T-cell depleted allogeneic human leukocyte antigen (HLA)-matched bone marrow transplant from her brother. Sixty-eight months following transplant, she was found to have relapsed and was treated with chemotherapy, donor stem cell boost, and donor lymphocyte infusion. Subsequently the patient had two additional relapses and was treated each time with gemtuzumab. Eight months following the second dose, she presented with fever, chills, sore throat, and tonsillar hypertrophy. Lymphadenopathy was observed in only the submandibular region, and a long-standing history of poor dentition was noted. CT imaging was negative for lymphadenopathy. Bone marrow biopsy showed relapse, and she received a third dose of gemtuzumab. PCR of whole blood was positive for EBV-DNA (18,000 copies/mL). Subsequent bone marrow biopsy showed plasmacytosis, without morphologic evidence of acute leukemia. Immunoperoxidase testing with EBV-latent membrane protein (LMP-1) antibody was positive in 50% to 70% of cells. Flow cytometric analysis of bone marrow was negative for clonal plasma cells. Serum protein was elevated, but no M spike was present. FISH evaluation of bone marrow was positive for XY chromosomal complement in 93.5% of cells, implying donor origin. The patient developed multiorganism infections and expired seven days following the final biopsy.

**Discussion:** We are unaware of another case of plasmacytic-type PTLD presenting in the bone marrow. Additionally, the time course of presentation (nearly eight years following transplant) and EBV positivity are unusual findings. Given the high percentage of cells with XY karyotype by FISH, donor plasma cells appear to be involved in the proliferation. Postulated mechanisms include the patient's continued immune suppression and superimposed EBV infection leading to PTLD. T-cell depletion of donor marrow (which this patient had) has been shown to represent a risk factor for the development of PTLD. Other risk factors, such as HLA-mismatched related donor and chronic graft-versus-host disease, were not present.

### 528 THE S GENE IN HEMORHEOLOGICAL PROPERTIES OF BLOOD IN NIGERIAN SMOKERS

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**Background:** The role of genes in the aetiology, diagnosis and therapeutic of disease is becoming more and more glaring in today`s medicine. This research study was aimed at investigating the role of hemoglobin S gene in hemorheological properties of blood in Nigerian smokers.

**Design:** A total of 256 apparently healthy volunteers, 70 Smokers, 56 Drinkers, 64 Non-Smokers and Non-Drinkers as controls and 75 Smokers and Drinkers together were studied. Their Packed Cells Volume(PCV), Hemoglobin concentration(Hb), Relative Plasma Viscosity(RPV), Relative Whole Blood Viscosity (RWBV), Plasma Fibrinogen Concentration(PFC), Hemoglobin-Genotype, Factor VII, Erythrocyte Sedimentation Rate(ESR) and Blood Pressure(BP) were analysed using reference methods.

**Results:** We observed a significant increase in PCV, Hb, PFC, ESR, RPV, RWBV and BP (ie diastolic and systolic pressure) ($P<0.005$) in Smokers alone,Smokers and Drinkers together compared to controls. However,those who smoke alone showed a significantly higher PCV, Hb, PFC, RWBV, Diastolic and Systolic pressure ($P<0.005$) than those who smoke and drink together. There were higher FVII, ESR, Diastolic and Systolic pressure ($P<0.005$) in Hb AA-genotype controls compared to Hb AS-genotype controls. A significant increase in PCV, Hb, PFC, RWBV, Diastolic and Systolic pressure ($P<0.005$)was found in Hb AA-genotype Smokers than those Smokers with Hb AS-genotype thus suggesting that Hb AS-genotype Smokers may be protected from arterial thrombosis.

**Conclusion:** We therefore conclude that Smokers have a higher hemorheological disturbance than Non-Smokers especially those Smokers who are Hb AA-genotype than Non-Smokers.

### 529 CORRELATION BETWEEN CHROMOSOMAL ABNORMALITIES AND IMMUNOHISTOCHEMICAL PROFILING IN DIFFUSE LARGE-B CELL LYMPHOMAS (DLBCL) REVEALS DISTINCT LYMPHOMAGENESIS PATHWAYS WITH CLINICOPATHOLOGIC SIGNIFICANCE AND PROGNOSTIC VALUE

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**Background:** DLBCL constitutes an heterogeneous group. The genetic and molecular mechanisms underlying their diverse clinical presentations and outcomes have been partially clarified by the recent application of DNA microarrays and tissues arrays technologies. Cytogenetic studies of DLBCL also revealed a broad spectrum of clonal genetic abnormalities and complex karyotypes, including chromosomal translocations, deletions, duplications and other undefined alterations. However the potential clinical relevance of these alterations and their links to pathology are still poorly defined .

**Design:** 101 previously untreated patients diagnosed with de novo DLBCL on lymph-node biopsies (98%) at our hospital between 1987 and 2003 were selected (median age = 59 years) .The inclusion criteria were the availability of appropriate paraffin- embedded-tissues and a karyotypic analysis using G-banding method. Hierarchical clustering analysis based on immunostaining with a large panel of antibodies (including cell-cycle proteins ,apoptosis, immune response and B-cell differentiation markers) was performed and correlated with recurrent cytogenetic abnormalities and clinical outcomes. The germinal center B-cell like (GCB) and the non-GCB phenotypes were defined using CD10, BCL6 and MUM1 .

**Results:** The GCB phenotype was observed in 46% of cases and is significantly related to t(14;18) (36%), trisomy 12 (36%), and 18q21 (45%) or 2p (31%) rearrangements. The non-GCB phenotype was observed in 54% of case and correlated to 3p (23%) and 3q (57%) rearrangements. DLBCL with t(14;18) were preferentially CD10+ (72%), BCL2+ (68%) and MUM1 negative (56%). By contrast, DLBCL with t(3;14) were more often p53+ (41%),

MUM1+ (94%), usually expressed the anti-apoptotic galectin-3 molecule (70%) but were BCL2 negative (88%). Using an unsupervised hierarchical clustering approach based on the expression of a large panel of antibodies, 82% of cases could be properly reclassified only by considering the presence of a t(14;18) or of a t(3;14), indicating clearly 2 distinct genetic basis of lymphomagénesis. P53 protein expression was correlated to 17p and 3q27 rearrangements. Clonal genetic abnormalities with a significant unfavourable prognosis impact were the 17p, 3p, 8q24 and 9p13 rearrangements. A scoring system, including all unfavourable genetic abnormalities was strongly predictive of the outcome(p:0,00016) and confirmed in an independent series of 87 DLBCL. In addition BCL2, CD5 and p53 expressions were associated to a poor clinical outcome (p:0,032;p:0,05;p:0,039 respectivly). Nuclear P16 expression and p53 expression had a predictive outcome worse than p16 alone (p:0,013) No association was found between GC/non-GC phenotype and clinical outcome.

**Conclusion:** This study demonstrates correlations between chromosomal abnormalities and immunohistochemical profiling in DLBCL. It also reveals distinct lymphomagenesis pathways with clinicopathologic significance and prognosis value.

**530 BLASTIC TRANSFORMATION OF MALT LYMPHOMA - A CASE REPORT**

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**Background:** Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue is a low grade mature B-cell neoplasm arising in mucosal sites of varies organs. It can transform to diffuse large B-cell lymphoma, a higher grade mature B-cell neoplasia. In contrast, precursor B lymphoblastic leukemia is a neoplasm of immature B-cells involving primarily bone marrow and peripheral blood. Most cases of B-ALL arise de novo. Only a small fraction of B-ALL cases arises from CML with blastic crisis. Rare cases transform from follicular lymphoma. No cases of B-ALL evolving from MALT lymphoma have been reported in the literature or encountered in our experience.

**Results:** A 65 year-old gentleman with history of orbital MALT lymphoma diagnosed in 2001 and treated with local radiation therapy with a good response, who two years later presented with a leukemic process composed of two distinct clonal B-cell populations in the peripheral blood and bone marrow as well ascites. One of them was composed of uniform, small lymphocytes whose phenotype was consistent with a marginal zone lymphoma (CD19+, CD20++, FMC-7+, HLA-DR+, CD25+, Kappa light chain+, CD34-, TdT-, CD5-, CD10- ). The second population had a blastic morphologic appearance and a phenotype characteristic for precursor B lymphoblastic leukemia (CD19+, CD34+, TdT+, CD38+, HLA-DR+, CD13+, CD20-, CD10-, Surface and cytoplasmic light chains- ). Cytogenetic studies performed with bone marrow showed a completx abnormal karyotype with 47,+XY,t(11;19)(q23;p13.3)[4]/50,XY,+3,+12,+18,+19[2]/46,XY[1]. After obtaining a brief response from induction chemotherapy for ALL, the patient relapsed with a dramatic increase in lymphoblasts. At this point, the mature B-cell population was not detected morphologically or immunophenotypically.

**Conclusion:** This patient with previous history of ocular adnexal MALT lymphoma developed precursor B-cell lymphoblastic leukemia with evidence of concurrent circulating marginal zone lymphoma cells. The clinical course is strongly supportive of lymphoblastic transformation of the preexisting MALT lymphoma, although the clonal relationship between these two neoplastic B-cell populations has not been confirmed at a molecular level. The disappearance of the marginal zone lymphoma cells shortly after induction therapy might be contributed to the therapy that had eradicated these cells or might indicate progression of the disease with complete clonal transformation to lymphoblasts. In summary, this is first case reported that likely represents a MALT lymphoma with lymphoblastic transformation. More cases with molecular studies are needed to confirm the above presumption and to exclude the possibility of de novo B-ALL from a new B-cell clone.

**531 DIFFUSE LARGE B CELL LYMPHOMA OF BONE MARROW AT INITIAL PRESENTATION**

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**Background:** Intravascular large cell lymphoma, Asian variant (AIVL) is recently proposed as a subtype of intravascular large cell lymphoma (IVL) with frequent bone marrow involvement of lymphoma cells and with infrequent skin lesion and neurological abnormalities. We experienced 37 cases of diffuse large B cell lymphoma of bone marrow (DLBCL-BM) at initial presentation and evaluated whether these cases were similar to AIVL or not.

**Design:** We examined clinicopathologically 37 patients with DLBCL-BM at initial presentation for the purpose of comparing with AIVL.

**Results:** Patients were 17 men and 20 women, with ages from 45 to 84 years (median 67). Clinically, patients had fever (24 cases), general fatigue (14 cases), anorexia (11 cases), dyspnea (2 cases), and hepatosplenomegaly (25 cases). Skin lesion (1 case), neurological abnormalities (4 cases), lymphadenopathy (7 cases), and no tumor mass formation were also observed. In laboratory data, elevated levels of lactate dehydrogenase were present in 34 cases. Hemophagocytic syndrome, which was diagnosed on the basis of clinical, laboratory and histopathologic criteria, were observed in 13 cases. Histologically, in bone marrow specimens, tumor cells are mainly non-cleaved cells with large nuclei and distinct nucleoli in most cases. These cells formed small to massive clusters or were scattered in bone marrow. Immunohistochemically, all cases were CD20 (L26) and except for one case, CD79a (mb-1) positive. We then evaluated the proportion of intrasinusoidal and extrasinusoidal lymphoma cells in bone marrow using factor ‡[ immunostaining. The neoplastic cells were observed in sinusoids in 8 cases, however, these cells predominantly infiltrated in extrasinusoidal spaces. In several cases, including autopsy cases (7 cases), the neoplastic cells infiltrated liver (6 cases), spleen (5 cases), kidneys (4 cases), lungs (3 cases), stomach (2 cases), and adrenal glands (1 case) with intravascular and/or diffuse infiltration pattern. In 9 cases, the neoplastic cells were found only in the bone marrow.

**Conclusion:** These results indicate that, in histological feature and infiltration pattern, these cases are different from IVL in which neoplastic cells proliferate only in the lumina of the small vessels. Mainly in East Asia including Japan, IVL cases were reported as Asian variant of IVL (AIVL). Our cases share some of the clinical features with AIVL, however, histologically neoplastic cells are present predominantly in extravascular space of the bone marrow and infiltration pattern is mainly in extravascular spaces. In conclusion we suggest these cases be regarded as primary bone marrow large B cell lymphoma.

**532 REAL-TIME QUANTITATIVE PCR DETECTION OF FUSION GENE NPM/ALK, CYTOKINE RECEPTOR CD30 AND T-CELL RECEPTOR REARRANGEMENT IN PATIENTS WITH ALCL: MONITORING RESIDUAL DISEASE AND CORRELATION WITH THE DISEASE STATUS**

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**Background:** Anaplastic large cell lymphoma (ALCL) represents a heterogeneous group of malignant lymphoproliferative diseases. Most of the cases are of T cell origin with a consistent expression of the cytokine receptor CD30 (Ki-1). ALCL is frequently associated with the t(2;5)(p23;q35) translocation fusing the anaplastic lymphoma kinase (ALK) gene and nucleophosmin (NPM) gene. The NPM/ALK chimeric gene encodes a constitutively activated tyrosin kinase that has been shown to be a potent oncogene. The NPM/ALK fusion protein is found in up to 75% of pediatric ALCL. The aim of the study was to monitor the minimal residual disease (MRD) using real-time quantitative reverse-transcription PCR (RQ-RT-PCR) of the NPM/ALK fusion gene and CD30 molecule, and real-time quantitative PCR (RQ-PCR) to detect clonal T-cell receptor rearrangement (TCR).

**Design:** RQ-RT-PCR and RQ-PCR are recently developed techniques for nuclear acid quantification. We prepared an assay using RQ-RT-PCR for the quantitative assessment of MRD in childhood ALCL by using a hydrolysis probe for quantification of NPM/ALK, LNA probe for quantification of CD30, and hybridization probes for quantification of the housekeeping gene (Beta-2 microglobuline gene), which was used as an internal cDNA quality and quantity control. We constructed plasmid standards with either NPM/ALK fusion gene and CD30 molecule or beta-2 microglobuline gene. Normalised expression of NPM/ALK and CD30 was determined as a ratio between NPM/ALK or CD30 and beta-2 microglobuline levels assessed by RQ-RT-PCR. We have used methodology of TCR and patient specific RQ-PCR according to European Biomed collaborative study.

**Results:** We analysed NPM/ALK, CD30 expression levels and clonal TCR rearrangement (TCR gamma, TCR delta) at diagnosis and/or relapses (cryopreserved and/or paraffine-embedded tissue) from affected lymph nodes in a cohort of 9 patients (4 – 34 years, median 13 years). In all of them hyperexpression of NPM/ALK and CD30 (median 7538 and 8735 copies, respectively) suitable for the MRD detection was detected. MRD level was analysed in 65 residual samples (bone marrow n=56, peripheral blood n=9). A combined analysis of the NPM/ALK levels, CD30 levels and corresponding TCR clonality and the course of disease showed a good correlation.

**Conclusion:** RQ-RT-PCR based analysis of NPM/ALK and CD30 expression is a promising and a rapid approach for monitoring MRD in patients with ALCL. However, the role of NPM/ALK and CD30 levels found as a residual disease needs further testing. Supported by grants from Czech Ministry of Health #00064203/6704, Czech Ministry of Education #0021620813 and Charles University GAUK #66/2004.

**533 MEDIASTINAL GERM-CELL TUMOR - HEMATOLOGIC MALIGNANCY SYNDROME WITH A RARE CYTOGENETIC FINDING**

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**Background:** Mediastinal germ cell tumor (GCT)-hematologic malignancy syndrome is a rare disorder. We report here a case of this syndrome with a rare histologic combination and a rare cytogenetic finding.

**Design:** A 16-year-old Japanese man presented with lumbago. The smear and clot section specimens and flow cytometry analysis of his bone marrow revealed the finding of acute myeloid leukemia (M7). The karyotype of bone marrow showed the near-triploid pattern and lack i(12p). Subsequently, a computed tomography (CT) scan examination disclosed the mediastinal tumor. He died of septic shock due to the bronchopneumoania and progressive liver failure.

**Results:** The post-mortem examination of the mediastinal tumor showed the combination of malignant teratoma (90%) and seminoma (10%).

**Conclusion:** The combination of seminoma in mediastinal tumor and near-triploid pattern without i(12p) in cytogenetic finding is very rare and this case may be informative in considering the pathogenesis of this syndrome.

**534 GENE EXPRESSION PROFILE OF TPM3-ALK POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA REVEALS OVERLAPPING AND UNIQUE PATTERNS WITH THAT OF NPM-ALK POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA**

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**Background:** Anaplastic large cell lymphoma (ALCL) comprises a group of non-Hodgkin

lymphomas characterized by the expression of the CD30/Ki-1 antigen. A subset of ALCL is characterized by chromosomal translocations involving the anaplastic lymphoma kinase (ALK) gene on chromosome 2. While the most common translocation is the t(2;5)(p23;q35) involving the nucleophosmin (NPM) gene on chromosome 5, up to 12 other translocations partners of the ALK gene have been identified. One of these is the t(1;2)(q25;p23) which results in the formation of the chimeric protein TPM3-ALK. While several of the signaling pathways induced by NPM-ALK have been elucidated, those involved in ALCLs harboring TPM3-ALK are largely unknown.
**Design:** In order to investigate the expression profiles of ALCLs carrying the NPM-ALK and TPM3-ALK fusions, we carried out cDNA microarray analysis of two ALCL tissue samples, one expressing the NPM-ALK fusion protein and the other the TPM3-ALK fusion protein. RNA was extracted from snap-frozen tissues, labeled with fluorescent dyes and analyzed using cDNAs microarray containing 9200 genes and ESTs. Quantitative fluorescence RT-PCR was performed to validate the cDNA microarray data on nine selected gene targets.
**Results:** There is significant overlap of genes deregulated in the NPM-ALK and TPM-ALK positive lymphomas. These deregulated genes are involved in diverse cellular functions such as cell cycle regulation, apoptosis, proliferation, and adhesion. Interestingly, a subset of the genes was distinct in their expression pattern in the two types of lymphomas. More importantly, many genes that were not previously associated with ALK positive lymphomas were identified.
**Conclusion:** Our results demonstrate the overlapping and unique transcriptional patterns associated with the NPM-ALK and TPM3-ALK fusions in ALCL.

### 535 PANCYTOPENIA DUE TO MASSIVE BONE MARROW INVOLVEMENT IN A PATIENT OF PRIMARY HYPEROXALURIA

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**Background:** Primary hyperoxaluria is a rare autosomal disorder characterised by overproduction and excessive urinary excretion of oxalate. This results in recurrent urolithiasis, nephrocalcinosis, and widespread deposition of calcium oxalate crystals, which is known as oxalosis. It is well-known that systemic oxalosis includes urolithiasis and nephrocalcinosis (kidney), bone pain, retinopathy (eye), cardiomyopathy and arrhythmias (heart), disseminated vascular lesions, neuropathies (nerve), synovitis (joints), subcutaneous calcinosis and livedo reticularis (skin).
**Design:** This case is presented because of its interesting clinical and morphologic features.
**Results:** A haemodialysed 21-year-old female, whose medical history revealed chronic renal failure, which was attributed to chronic obstructive nephropathy due to primary hyperoxaluria, presented with pancytopenia. She also suffered from bone pain. A bone marrow biopsy was undertaken to illuminate the aetiology of the pancytopenia. Bone marrow biopsy revealed numerous birefringent crystalline deposits filling almost all the intertrabecular medulla. Giant cells of foreign type were lining crystalline deposits, which were not stained with H&E. The rest of the medulla showed a reactive hyperplasia in all cell lines. Liver biopsy, which was simultaneously performed, revealed only grade IV haemosiderosis and extramedullary haematopoiesis.
**Conclusions:** Anemia is an important cause of morbidity in patients suffering from chronic renal failure and it is usually treated by erythropoietin. But, the aetiology of the renal failure plays an important role for determining the treatment of anemia in a such case. However, pancytopenia from bone marrow infiltration of oxalate crystals is a rare complication of primary hyperoxaluria. The kidney transplantation alone has a lower survival rate because defective enzymes are normally found in the peroxisome (alanine glyoxalate aminotransferase) or in the cytoplasm (glyoxalate reductase) of hepatocytes. Although it has been shown that a case presenting with pancytopenia from bone marrow involvement reversed following kidney transplantation, a combined liver and kidney transplantation with bone marrow transplantation is planned in our patient. Furthermore, the 10 year survival rate after combined transplantation has been reported as 70%. The patient is alive with symptomatic treatment modalities and is still waiting for a convenient donor.

### 536 CLINICO-PATHOLOGICAL ANALYSIS OF SPLENECTOMY SPECIMENS

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**Background:** The spleen can be a difficult specimen for the surgical pathologist as there is often discordance between the patient's clinical condition and the pathological findings. Splenectomy is typically used as a therapeutic procedure; however, in a subset of cases it is done with diagnostic expectation. Histopathological examination of the spleen may identify unexpected findings; however, there are very few studies on surgical pathology of splenectomy specimens. The present study was undertaken to analyze pathological findings in splenectomy specimens and correlate them with the clinical findings.
**Design:** A total of 68 splenectomy specimens received during the period from1998 to 2006 comprised the study material. The clinical details were retrieved from the patients' record files. Hematoxylin and eosin stained paraffin sections were studied; histochemical and immunostains were performed wherever required.
**Results:** In the present series, indication for splenectomy was therapeutic in 42 cases (61.7%) and diagnostic splenectomy in 21 cases (30.8%), while incidental splenectomy as part of other abdominal surgery was performed in 5 cases (7.3%). On pathological examination, splenic lesions were broadly divided into two categories: neoplastic (5 cases, 7.3%), and non-neoplastic (63 cases, 92.6%). In the neoplastic group, there was one case each of primary non-Hodgkin's lymphoma, Hodgkin's lymphoma, leukemic infiltration, angiosarcoma and cavernous hemangioma. In the non-neoplastic group, majority of the cases were of traumatic rupture (37 cases, 58.7%); other lesions seen were chronic venous congestion (12 cases, 19%), inflammatory pathology (8 cases, 12.6%) (e.g. splenic abscess, malarial spleen, tuberculosis and fungal infection), splenic cysts (5 cases, 7.3%) and one case showed extra-medullary hematopoiesis.
**Conclusion:** Splenectomy specimens present a broad spectrum of pathological findings of varied surgical importance. In our settings, non-neoplastic traumatic/ infectious lesions were more frequent than neoplastic lesions. In a subset of patients, option of diagnostic surgical procedure with conservation of the spleen can be offered.

### 537 ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA, COMPLICATED BY PROLIFERATION OF LARGE B-CELLS IN THE LYMPH NODE AND BONE MARROW- A POTENTIAL DIAGNOSTIC PITFALL,

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**Background:** Angioimmunoblastic T-cell lymphoma (AITL) is an aggressive lymphoma representing about 1-2% of all types of Non-Hodgkin`s Lymphoma. Presence of large B cells in AITL has previously been described in the literature. The B-cell expansion in this lymphoma is often accompanied by EBV expression.
**Design:** We report a case of 70-year-old male with previous diagnosis of multiple myeloma treated with VAD chemotherapy and peripheral autologous stem cell transplantation. He now presented with progressive diffuse lymphadenopathy as well as a lung lesion. A bone marrow biopsy and left groin lymph node biopsy were performed as per lymphoma protocol and fresh tissue was submitted for flow cytometry and in formalin for histopathological assessment. An appropriate panel of immunostains was performed on both biopsies using Standard Labelled Strept Avidin Biotin technique (LSAB).
**Results:** The bone marrow biopsy showed a hypercellular bone marrow with interstitial infiltration of medium- sized lymphoid cells admixed with eosinophils, plasma cells and large blastic cells. Immunohistochemically, these large cells were positive for CD20, CD79a, bcl-2 and bcl-6 with the smaller lymphoid cells being positive for CD3 and focally for CD10. Kappa and lambda light chains highlighted admixed polyclonal plasma cells, which were negative for CD56. The lymph node showed marked interfollicular expansion by small and medium to large cells admixed with eosinophils, few plasma cells and numerous arborizing venules. Residual lymphoid follicles were pushed or compressed to the periphery. The interfollicular zones also showed clusters of large blastic looking cells. Immunohistochemistry revealed that atypical interfollicular cells were predominantly T cells expressing CD3, CD4, CD7 with reduced expression of CD5 and CD8 and variable positivity for CD10. CD20 and CD79A highlighted residual follicles as well large cells in the interfollicular area. These large cells also stained for Bcl-2. In-situ hybridization by EBER probe was positive for EBV. Flow cytometry and PCR revealed a monoclonal T-cell population with polyclonal B-cells both in the bone marrow and lymph node.
**Conclusion:** The presence of large number of blastic looking B-lymphocytes in the interfollicular zones of the lymph node and presence of similar cells in the bone marrow with positive staining for CD20, CD79A, Bcl2 and EBV could be misdiagnosed as diffuse large B cell lymphoma (DLBCL) in AITL. The large cells can predominate in some cases and camouflage the neoplastic T –cell population. Presence of follicular dendritic cell proliferation around the arborizing blood vessels, CD10 positive T-cell expansion, and awareness of presence of these large polyclonal B-cells prevents this potential diagnostic pitfall with DLBCL. Clonality studies are also useful as the large B cells are polyclonal. Patient surveillance and follow-up is mandatory, as small percentage can progress to B cell lymphoma in the setting of AITL.

### 538 AN UNUSUAL PRESENTATION OF LANGERHANS' CELL SARCOMA IN A BACKGROUND OF LOW GRADE FOLLICULAR CENTRE CELL LYMPHOMA

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**Background:** Tumours of histiocytic and accessory dendritic cells are relatively rare and difficult to diagnose. Recently, International Lymphoma Study Group (ILSG) has proposed five categories based on immunohistochemistry principally: histiocytic sarcoma, langerhans' cell tumours, langerhans' cell sarcoma, follicular dendritic cell tumours/sarcoma and interdigitating cell tumours/sarcoma. Occurrence of these tumours in association with other malignancy is extremely uncommon. An unusual case of Langerhans' cell sarcoma is presented.
**Design:** The case of Langerhans' cell sarcoma is described that arose in a background of low grade follicular centre (FCC) B cell lymphoma of nine year duration. Diagnostic features are discussed.
**Results:** A 60-year-old male was admitted with fever and rapidly enlarging mass in the right axilla of one week duration. He was diagnosed stage IV low grade follicular centre cell lymphoma of B cell type in 1996. He was asymptomatic with non-progressive clinically small volume disease of the axillae and groins and required no treatment. The MRI scan done showed mass encasing the axillary vessels and extending to the chest wall. The biopsy of the axillary mass was fragmented consisting of lymph node with distended sinuses and distorted architecture. The sinuses showed sheets of cells consisting of large cells with abundant eosinophilic cytoplasm and pleomorphic nuclei containing nucleoli and irregular chromatin. A panel of markers was carried out and showed these cells to be negative for CD3, CD20, CD79a, CD5 and CD30. They were found to be positive for S100, CD1a, and CD 68 and focally for CD4 and CD15. This co-expression of S100 and CD1a confirmed the Langerhans' cell differentiation. There was residual low grade FCC lymphoma in the perinodal fatty tissue. The bone marrow biopsy was involved by FCC lymphoma but not Langerhans' cell sarcoma. The disease was rapidly and fatally progressive despite combination chemotherapy.
**Conclusion:** We have presented a rare case of langerhans' cell sarcoma arising in an untreated FCC lymphoma. The diagnosis of this rare entity could only be established with use of immunohistochemistry with demonstration of co-expression of S100 and CD1a as the morphology was not typical. Only one previous case of Langerhans' cell neoplasm has

been reported in association with FCC lymphoma to the best of our knowledge. Whether this represents a transformed or second malignancy is not clear.

**539 SUBCUTANEOUS PANNICULITIS-LIKE T-CELL LYMPHOMA PRESENTING IN VISCERAL ADIPOSE TISSUE WITHOUT CUTANEOUS LESIONS: A CASE STUDY AND LITERATURE REVIEW**

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**Background:** Subcutaneous Panniculitis-like T-Cell Lymphoma (SPTL) is a rare lymphoma of cytotoxic T-cells which preferentially infiltrates subcutaneous tissue, generally without extracutaneous involvement. Since its earliest recognition in 1991, documented cases of SPTL almost invariably present with clinically apparent subcutaneous-centered cutaneous lesions. Rare atypical cases have been reported including one case that initially presented as Bronchiolitis Obliterans Organizing Pneumonia, and another with a leukemic transformation of a typical SPTL. In general, SPTL is considered an indolent disease although it may occasionally follow an aggressive and rapidly fatal course due to the evolution of a hemophagocytic syndrome. A terminal hemophagocytic syndrome was reported in one case of SPTL that appeared to be in complete remission without clincial evidence of disease. Although the lipotropism of the neoplastic cells in SPTL appears to selectively target the subcutis, there are limited autopsy reports in the literature, and no papers addressing whether SPTL affects other adipocytic sites.

**Design:** For this case study, information was obtained through a thorough review of the patient's chart, and from performance of the autopsy with ancillary immunohistochemical and molecular studies.

**Results:** Here we present the case of a 40 year old female admitted to hospital with fever of unknown origin and abdominal pain. Despite extensive in-patient investigations, the patient's underlying illness went undiagnosed prior to her death. Ultimately, the patient developed a hemophagocytic syndrome with evolving liver failure, and died three weeks after admission due to a bleeding diathesis. Cutaneous lesions were not identified clinically or upon post-mortem examination. Autopsy revealed diffuse and widespread infiltration of the intraperitoneal, retroperitoneal and peri-cardiac fat by small, mildly to moderately atypical lymphocytes showing characteristic rimming of adipocytes with panniculitis, karyorrhexis, and cytophagocytosis. Polymerase chain reaction amplification demonstrated monoclonality of the T-cell population which exhibited a CD3+, CD4-, CD56-, CD8+ immunophenotype upon immunohistochemistry. Hemophagocytosis was evident throughout the reticulo-endothelial system. The liver showed severe macrosteatosis.

**Conclusion:** The current WHO-EORTC definition for SPTL classifies it as a cutaneous lymphoma which preferentially infiltrates subcutaneous tissue, primarily over the extremities and trunk, and generally without extra-cutaneous involvement. The literature supports this definition with nearly all documented cases demonstrating characteristic skin lesions and only rarely with secondary extra-cutaneous involvement. To our knowledge, the literature does not report SPTL involvement of visceral adipose tissue. In addition, SPTL with occult or absent cutaneous disease is an exceedingly rare presentation. Our case demonstrates a unique presentation of SPTL involving visceral adipose tissue without evident cutaneous lesions. This case suggests that the lipotropic nature of SPTL cells may not be limited to the subcutis. Further studies are required in order to determine whether the neoplastic T-cells in SPTL commonly infiltrate adipose tissue at other sites.

**540 CELLULAR RETINOL- BINDING PROTEIN-1 (CRBP-1) IS MODULATED IN STROMAL MYOFIBROBLASTS AND MEGAKARYOCYTES IN CHRONIC MYELOPROLIFERATIVE DISORDERS**

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**Background:** The effects of retinol (ROL) are mediated by cytoplasmic binding proteins involved in retinoid transport and/or metabolism, as well as nuclear receptors which act as ligand-dependent transcriptional regulators. Cellular retinol-binding protein (CRBP) -1 contributes to the esterification of ROL to retinyl esters, the oxidation of ROL to retinal, the hydrolysis of retinyl esters into ROL. It also has been implicated in cellular growth and differentiation. Heterogeneous patterns ranging from over-expression of CRBP-1 to down-regulation via epigenetic silencing through DNA hypermethylation has been reported in several malignancies. To investigate the involvement of this key protein of retinoid homeostasis and metabolism in myeloproliferative diseases (MPD), we analysed the in situ expression patterns of CRBP-1.

**Design:** This study was performed on a cohort of healthy bone marrow donors (n=15), patients with essential thrombocythemia (ET; n=25), chronic idiopathic myelofibrosis (CIMF; n=25) and polycythemia vera (PV; n=25) in accordance with the local ethics policy. Diagnoses of ET, CIMF and PV were strictly in accordance with the clinical, morphological, laboratory and cytogenetic features established by the World Health Organization Classification. The tissue localization of CRBP-1 in marrow trephines was visualized using brightfield and confocal laser scanning microscopy (CLSM). Double-labeling experiments included a panel of additional antibodies such as CD61, CD34 or á-smooth muscle actin (SMA) and the intermediate filament protein synemin. Semiquantitative evaluation focused on CRBP-1 expression in megakaryocytes and bone marrow stromal cells/myofibroblasts (MSCs/MFs).

**Results:** CRBP-1 positive MSCs/MFs were present in subsets of MPD patients, but not in normal controls. Colocalization of CRBP-1 and SMA was documented by CLSM. The up-regulation of CRBP-1 in MSCs/MFs was associated with an increased fibre density in the various MPD entities including CML, CIMF and PV, but not in ET. Megakaryocytes from healthy control persons showed a moderate to high cytoplasmic CRBP-1 immunoreactivity. In contrast to the stroma, heterogeneous, but generally decreased levels of CRBP-1 expression were demonstrated in CD61-positive megakaryocytes of PV, CIMF and subsets of ET. CRBP-1 loss or abnormal spotty plasmalemmal localization was most prominent in the bizarre giant megakaryozytes of CIMF.

**Conclusion:** The increase of CRBP-1, a protein involved in retinoid metabolism, is considered as a marker of acivation of granulation tissue fibroblasts when they differentiate into myofibroblasts. The modulation of CRBP-1 in MSCs/MFCs of the bone marrow microenvironment may affect proliferation, migration, differentiation, matrix synthesis and turnover, and influence Vitamin A homeostasis in MPD. Moreover, our data implicate that the retinoid-signaling pathway may be impaired in the megakaryocytes of MPD patients. The down-regulation of CRBP-1 in megakaryocytes may have potent effects on genes which regulate differentiation. Thus, our observations further suggest a link between a modulation of CRBP-1 expression and carcinogenesis, which is in accordance with previously published studies on various human tissues.

**541 ADULT AND PEDIATRIC HODGKIN IN EGYPT: IMMUNOPHENOTYPE, EBER EXPRESSION AND B-CELL DIFFERENTIATION**

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**Background:** Hodgkin`s lymphoma (HL) differs from non-Hodgkin`s lymphoma(NHL) in its bimodal presentation. Whether adult differs from pediatric Hodgkin in its histological type, immunophenotype,presence or abscence of EBV infection, is still a subject of debate.

**Design:** To further elucidate this problem , we studied 62 cases of Classical Hodgkin`s lymphoma (CHL):28 children and 34 adolescents for the immunohistochemical expression of CD30, CD15, CD45, Pax-5, and CD138 (Syndecan-1) using both the standard peroxidase and APAAP techniques .Moreover, The Epstein Barr Virus Encoded RNA (EBER) expression was studied using the in situ hybridization technique . All studies were done on paraffin embedded ,formalin-fixed material.

**Results:** In the pediatric group, the positive expression of CD15, CD30, CD45, CD20, CD138 was 60.7%,100%,7.1%, 28.6%, and 64.3% respectively. In adult group the expression was 50%, 94.1%, 17.6%, 17.6% and 29.4% respectively. Pax-5 (BSAP) was 100% positive in both groups.EBER expression was found in 50% of pediatric and 20% of adult cases. In the adult group, CD15 expression positively correlated with both Syndecan and EBER expression (p=0.019 and p=0.009, respectively).In the pediatric group, EBER expression was more in younger age (p=0.007).

**Conclusion:** Pathogenesis of pediatric Hodgkin seems to be different from adult HL, the Epstein Barr virus possibly being the initiating event . The expression of Syndecan and its positive correlation with CD15 ( a previously reported good prognostic factor) could define a subset of CHD with a switch into the immunoglobulin secreting phenotype ,with a better prognostic potential.

**542 MUM1 EXPRESSION IN FOLLICULAR LYMPHOMA IS NOT PREDICTIVE OF PATIENT OUTCOME**

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**Background:** Follicular lymphoma (FL) is the second most common lymphoma in North America, and has a diverse spectrum of clinical behavior. There are very few biologic prognostic markers predictive of overall survival. A recent study suggested that MUM1 expression identified a group of patients with poor overall survival (Blood 2005; vol. 106; page 291a). The objective of this study was to determine the prognostic relevance of MUM1 expression in a group of patients with FL that were uniformly treated.

**Design:** Between 1987 and 1993, the BC Cancer Agency enrolled 126 patients with FL on a phase II study utilizing BP-VACOP and involved field radiotherapy. All patients were treatment-naive, <61 years of age, and had advanced-stage FL. Paraffin blocks were available for tissue microarray (TMA) construction from 105 of these patients. The TMAs consisted of duplicate 1.0mm cores of diagnostic biopsies and were all screened with CD20 antibodies to ensure tumor cell content. The TMAs were immunostained for MUM1. Cases were considered positive if 10% or greater of cells showed nuclear staining. In addition, we used the publicly available FL gene expression database of the Lymphoma/Leukemia Molecular Profiling Project (LLMPP) consortium (http://llmpp.nih.gov) to test the prognostic value of MUM1 gene expression in FL. Four transcripts of MUM1 are present on the U133 Affymetrix arrays used in the LLMPP study. The expression of the MUM1 transcripts were averaged for each of the 191 FLs, data divided into quartiles, and correlated with patient outcome.

**Results:** In the TMA, 102/105 cases were successfully arrayed and comprise the study group. 35/102 (34%) cases were positive for MUM1 by immunohistochemistry. MUM1 expression was not predictive of overall survival or progression-free survival. The gene expression of MUM1 from the LLMPP study also did not correlate with patient outcome.

**Conclusion:** MUM1 protein expression is not predictive of overall survival in a group of uniformly aggressively treated patients with FL.

**543 LEUKEMIC PHASE ANAPLASTIC LARGE CELL LYMPHOMA**

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**Background:** Anaplastic large cell lymphoma (ALCL) is an aggressive neoplasm of T- or null cell phenotype that comprises 10-15% of non-Hodgkin lymphomas in children and approximately 3% of non-Hodgkin lymphomas in adults. Leukemic phase ALCL has rarely been reported in the literature. Most cases with leukemic involvement are the small cell variant of ALCL. These cases often lack the pleomorphism seen in the common variant of ALCL and may be misdiagnosed.

**Design:** We report a series of three patients who presented with leukemic phase ALCL. The patients included an 11-year-old boy, a 29-year-old man and 59-year-old woman. These patients had pathologic specimens reviewed at the University of Michigan between 2002 and 2006. The clinical and pathologic features of these cases are reviewed in detail.

**Results:** Our series of patients with leukemic phase ALCL exhibited a multitude of rare clinical features. These findings included documented CSF involvement in two patients and mental status changes in the third. Two patients also had documented sinusoidal and mild periportal hepatic involvement by liver biopsy. Two of the patients had small cell variant ALCL. The neoplastic cells in all three patients were ALK positive with t(2;5) translocation demonstrated by cytogenetics. In spite of the ALK positivity, two of the patients died within months of diagnosis.
**Conclusion:** The leukemic phase of ALCL is rare and behaves in an aggressive manner. Some, but not all, cases reported in the literature presenting with peripheral blood involvement had the small cell variant, as seen in two of our cases. Multi-system organ involvement, including liver and CSF, was found in our series. CSF involvement was documented morphologically as well as by flow cytometry. The leukemic phase of ALCL should be considered when a leukemia with unusual morphologic features is encountered.

**544 MOLECULAR LINEAGE ANALYSIS IN THE DIFFERENTIAL DIAGNOSIS OF HEMATOLYMPHOID NEOPLASIA--A STUDY OF 31 CASES**
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**Background:** By current WHO criteria, most—though not all—cases of hematolymphoid neoplasm can be diagnosed immunomorphologically, diminishing the role of molecular testing for lymphoid antigen receptor clonality in lymphoma diagnosis. Hence, our objective was to glean immunomorphological and molecular correlates from hematolymphoid neoplasms that had remained unresolvable without diagnostic molecular input.
**Design:** Thirty-one such cases were reviewed histologically and with standard immunoperoxidases. In-situ hybridization for Ebstein-Barr virus (EBV)-encoded RNA (EBER) was performed on selected cases. Polymerase chain reaction (PCR) of T-cell receptor (TCR) and immunoglobulin heavy chain (IgH) genes was performed on whole tissue in all cases and on microdissected cells in two.
**Results:** Twenty-three (74%) of our cases had some form of peripheral T-cell lymphoma (PTCL), of which 18 (78%) were complicated by a proliferation of B-lineage cells, either within the same tissue ("syntopic") as large B-cells (LBC) or Reed-Sternberg (RS)-like cells (15 cases, transient in 2), florid lymphoid hyperplasia (2 cases, one also with syntopic LBC) or monotypic plasma cells (1 case), or at a separate ("metatopic") site as a B-cell lymphoma (2 cases, one of which also had syntopic LBC) or Hodgkin lymphoma (HL, 1 case, also showing syntopic LBC). Eight (44%) of these 18 PTCLs with B-lineage proliferation fulfilled current immunomorphological criteria for categorization into the angioimmunoblastic subtype, and 16 (89%) yielded monoclonal TCR gene rearrangements, inclusive of the only two (12%) that showed IgH clonality, which was transient in one case. Three (17%) of them had originally been misinterpreted as some form of HL. Conversely, of the remaining cases, 4 (80%) out of 5 that had been diagnosed initially as some form of large cell non-HL (NHL), including 3 out of 4 that were called "anaplastic", had to be revised to Grade II/syncytial nodular sclerosing (NS) HL, yielding polyclonal TCRγ gene rearrangements, with 2 cases, in addition, disclosing neoplastic cell nuclear positivity for B-cell-specific activator protein/pax-5 gene product and yielding clonal IgH gene rearrangements, thereby excluding anaplastic large cell lymphoma according to current WHO criteria.
**Conclusion:** Paradoxically, monoclonality of TCR rather than IgH gene rearrangement may more often be detectable with a predominantly dispersed ("Hodgkinoid"), large B-lineage cell proliferation, consistent with release from immune regulation in the milieu of impaired immunosurveillance within a PTCL. This is further compounded by the difficulty in ascertaining clonal IgH gene rearrangements due to the high prevalence of somatic hypermutations that interfere with consensus primer hybridization, as well as "dilution" in a T-cell-rich milieu—the same reason for the long-elusive lineage of RS cells in HL. Conversely, anaplastic lymphoma, which is of non-B-lineage, may often be mimicked by NSHL, which is of B-lineage.

**545 CASE REPORT OF A BCL6 TRANSLOCATION IN PROGRESSIVE TRANSFORMATION OF GERMINAL CENTERS**
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**Background:** PTGC is a pattern of lymph node reactive hyperplasia. It can also be the predominant pattern in hyperplasic lymph nodes: florid PTGC. It is characterized histologically by the expansion of mantle zone lymphocytes within adjacent sinusoids and germinal centers. The lymphocytes are predominantly B-cells. Morphologically, it can be mistaken with NLPHD because of its nodular pattern and because of the presence of large cells that can be misidentified as L&H cells. Furthermore, PTGC has been considered to represent a precursor lesion of NLPHD, but the relationship between PTGC and NLPHD remains unclear. Here we report a surprising case of PTGC arising in a cervical lymph node of a 12 years old boy with a translocation 3;22 discovered by cytogenetic analysis. This BCL6 translocation has been described in diffuse B cell lymphomas and cases of NLPHD but not in PTGC.
**Design:** A 12 year old boy was referred to our hospital for the investigation of an asymptomatic single, enlarged cervical lymph node. He was an otherwise healthy young boy without any past history of lymphoma in his personal records or in his family. A lymph node biopsy was performed and a cytogenetic and FISH study was made.
**Results:** Our case is macroscopically and morphologically typical of PTGC. However, a cytogenetic study has revealed a translocation of the BCL6 oncogene, t(3;22). Our FISH study failed to confirm the translocation.
**Conclusion:** In our final report, we concluded that it was a very unusual case of PTGC owing to the presence of a translocation of the BCL6 oncogene t(3;22) in the karyotype. This unique case of PTGC associated with a BCL6 translocation might represent a molecular proof in favour of the relationship between PTGC and NLPHD. Further studies are underway to confirm this hypothesis.

**546 PREVALENCE OF LYMPHOMA SUB-TYPES IN SHANXI OF CHINA ACCORDING TO LATEST WHO CLASSIFICATION**
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**Background:** Quite dramatic differences in the frequency of various types of malignant lymphoma have been demonstrated between different countries.
**Design:** To analyze the prevalence of lymphoma subtypes in Shanxi of China according to the latest WHO classification, and to compare the figures with those in other parts of the world.The hematoxylin and eosin-stained sections of 447 lymphoma cases from the archive files of Shanxi Tumor Hospital were reviewed. Immunohistomchemical study was performed using a panel of antibodies, including ALK1, bcl-6, CD (1a, 3, 4, 5, 7, 8, 10, 15, 20, 23, 30, 43, 56, 68, 79a and 99), cyclin D1, EMA, IgD, kappa, lambda, LMP1, PAX5, Tdt and Vs38C. In addition, in-situ hybridization for Epstein-Barr virus-encoded RNA (EBER) was carried out. All cases were then reclassified according to the latest WHO classification of lymphoma.
**Results:** Of the 447 cases studied, 385 cases (86.1%) were confirmed to be non-Hodgkin lymphoma (NHL), while 62 cases (13.9%) belonged to classic Hodgkin lymphoma (HL). Of the NHL cases, 68.3% were of B-cell lineage and 30.6% were of T and/or NK-cell lineage. Histiocytic neoplasm accounted for only 0.8% (3 cases). As for the subtyping of NHL, diffuse large B-cell lymphoma was commonest (35.1%), followed by peripheral T-cell lymphoma (12.0%), extranodal marginal zone B-cell lymphoma (MALT lymphoma) (11.7%), follicular lymphoma (8.6%), T-lymphoblastic lymphoma (7.0%), anaplastic large cell lymphoma (4.2%), B-small lymphocytic lymphoma (3.6%) and mantle cell lymphoma (2.6%). Amongst the 263 cases of B-cell lymphoma, 105 cases (39.9%) expressed immunoglobulin light chain (kappa in 52 cases and lambda in 53 cases) in paraffin sections. Regarding markers for EB virus infection, 14 cases of the B-cell lymphoma gave positive findings with both EBER in-situ hybridization and LMP-1 immunohistochemistry, while 6 of the T/NK-cell lymphoma expressed LMP-1 and 19 showed positive signals for EBER. In NHL, there was discordance in EBER in-situ hybridization and LMP-1 immunohistochemical results. As for HL, EB virus positivity was noted in 37 of the 62 cases (59.7%), including 7 cases of lymphocyte-rich HL, 11 cases of mixed cellularity HL and 19 cases of nodular sclerosis HL. In classic HL, there was complete concordance of results by both EBER in-situ hybridization and LMP-1 immunohistochemistry.
**Conclusion:** The prevalence of diffuse large B-cell lymphoma in Shanxi of China is similar to that in western countries and other parts of Asia. The incidence of follicular lymphoma however is much lower. On the other hand, T/NK-cell lymphoma is more common, when compared with other Asian countries including Japan and Korea.

**547 LARGE CELL TRANSFORMATION OF MYCOSIS FUNGOIDES IN LYMPH NODES: A CLINICOPATHOLOGIC STUDY BY FLOW CYTOMETRY AND FINE NEEDLE ASPIRATION BIOPSY**
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**Background:** Large cell transformation (LCT) in mycosis fungoides (MF) is rare and is associated with an aggressive clinical course. LCT of MF can occur in the skin or extracutaneous sites such as lymph nodes (LN) and is defined by the presence of large cells in more than 25% of the infiltrate. The diagnosis of LN involvement in MF including LCT can be accomplished by an excisional biopsy of the LN or fine needle aspiration biopsy (FNAB) in conjunction with flow cytometry.
**Design:** We retrieved specimens from 19 patients with a diagnosis of MF presenting with lymphadenopathy suspicious for MF involvement from the archives of Department of Pathology from our institution. All the patients had at least one FNAB of an enlarged LN submitted for flow cytometry. All cases were analyzed using two or four color flow cytometry and antibodies for CD2, CD3, CD4, CD5, CD7, CD8, CD19, kappa and lambda immunoglobulin light chains. In cases with adequate material, CD25, CD38, CD40L, HLA-DR, TCRalphabeta and TCRgammadelta were also tested. We also analyzed the bone marrow (BM)(4 cases), peripheral blood (PB)(15 cases) and cerebrospinal fluid (CSF)(1 case) by flow cytometry. The FNAB smears were reviewed to determine large cell transformation. PCR studies for gammaTCR gene were performed in 29 samples (13 PB, 10 skin, 6 LN) from 15 patients.
**Results:** There were 10 female and 9 male patients, ranging from age 39 to 79 yrs (median 68). The LN-FNAB of 18/19 patients were diagnostic and showed MF involvement by flow cytometry and cytology. The FNAB smears showed an increased number of large cells in 8/18 cases (42%), consistent with LCT. The LCT cases (n=8) revealed most commonly CD4-/CD8-(4/8); and in one of these cases subsequent testing revealed a CD4+/CD8+ phenotype. Overall, 4/8 cases (50%) with LCT in the LN showed immunophenotypic change over time in different body sites (PB, CSF). Less common immunophenotypes in the LCT group were: increased CD4/CD8 and CD7-(2/8), increased CD4/CD8 only(1/8), HLA-DR+(1/6), CD25+(0/2), CD38+(1/2) and CD40L+(1/1). These immunophenotypes were observed in non-LCT cases (n=10): increased CD4/CD8 and CD7- (6/10), increased CD4/CD8 (3/10), normal CD4/CD8 and CD7- (1/10), HLA-DR+(4/7), CD25+(1/4), CD38+(1/1), CD40L+(0/1), CD20 co-expression (1/10). Other body sites involved included PB(4), CSF(1), testis(1), salivary gland(1) in the LCT group and PB(6) and BM(1) in the non-LCT group. Two unexpected lymphomas were detected in the non-LCT group in addition to MF involvement; mantle cell lymphoma (1) and small lymphocytic lymphoma (CLL/SLL) (1).
**Conclusion:** Flow cytometry in conjunction with FNAB can successfully detect LN involvement in MF including large cell transformation. The immunophenotype of the LCT cases is distinctly different than the non-LCT cases and immunophenotypic changes

are commonly seen in LCT in lymph nodes. Flow cytometry is critical to diagnose other unexpected lymphomas in patients with MF involving LNs.

## Hepatobiliary/Pancreas

### 548 PATHOMORPHOLOGICAL FEATURES OF THE LIVER POST-BONE MARROW TRANSPLANTATION IN MURINE SCHISTOSOMIASIS

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**Background:** Treatment of liver harm by bone marrow (BM) stem cells is a dream to all scientists working in that field particularly when they think of the very long waiting lists for liver transplants.

**Design:** This work was designed in order to study the pathomorphological features in the recipient liver and intestine of female mice, chronically infected with schistosomiasis mansoni and transplanted by un-fractionated bone marrow cells (BM) from healthy male mice of the same strain. Our aim was to identify and follow up the presence and differentiation of the transplanted BM cells into hepatic and bile ductular cells as well as the consequent histopathological hepatic morphological features exerted by the transplanted cells. Material and Methods of this work comprised non ablated female albino mice, that were classified into the following subgroups; one group infected with schitosoma mansoni , one group infected with schistosoma mansoni and transplanted with BM cells, one normal control mice group and transplanted with BM cells and the last one is a normal control mice group. BM cells were injected at 14 weeks post infection, via two routes in each group; intrahepatic and intravenous. Mice started to be sacrificed two weeks post-BM injection at one week intervals (from 16-29th week post infection).

**Results:** The studied paraffin sections from both liver and large intestine of infected transplanted group revealed some morphological changes in comparison to both control groups (normal transplanted and infected). These changes include: focal appearance of small scattered young hepatocytes exhibiting small rounded centrally located nuclei surrounded by eosinophilic cytoplasm mainly detected at periportal areas. There are also scattered primitive and incomplete forms of small groups of bile ductules within portal tracts lined by primitive epithelial cells exhibiting abundant effaced faint pink cytoplasm and central smaller nuclei than normal. There is also relative increase in the newly formed small blood vessels especially surrounding the periovular schistosomal granulomas in both hepatic and intestinal sections . Electron microscopic studies of the liver sections from the infected transplanted group revealed a relative increase in hepatic regenerative changes. Donor BM derived cells showed y-chromosome by FISH technique performed on unstained paraffin liver tissue sections. CDYL protein and Albumin were also recovered in the infected transplanted livers by using the indirect immmunofluorescence technique.

**Conclusion:** Our data revealed differentiation of some of the injected BM stem cells into few scattered young hepatocytes, foci of primitive bile ductules and possibly small newly formed blood vessels especially more recorded within the groups sacrificed 2-4 weeks post BM injection via the direct intrahepatic injection route. Much more extensive studies are required in order to verify the real ability of the BM stem cells to fully differentiate morphologically and functionally into healthy hepatic tissue.

### 549 SPECIFICITY OF C4D IMMUNOSTAINING IN PANCREAS ALLOGRAFT BIOPSIES

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**Background:** C4d is a well-established marker for humoral rejection in renal allografts and we have experience with C4d staining being indicative of humoral rejection in pancreas allografts. However, the specificity of C4d staining has not been determined. The purpose of this study is to attempt to determine the specificity of C4d staining in pancreas by performing C4d staining in allograft and non-allograft pancreas specimens.

**Design:** This represents a review of all pancreatic allograft biopsies and explants from the transplant program of UMDNJ- Robert Wood Johnson Medical School as well as prospective staining of more recent specimens. The specimens were diagnosed for acute cellular rejection, chronic rejection and /or other pathologic processes (ischemia, infection, etc.). The control population consisted of pancreas specimens removed for pathology unrelated to transplant; predominantly pancreatic adenocarcinoma. Immunohistochemical staining with antibodies towards C4d (ARPTM Belmont MA, rabbit polyclonal antibody) was performed on a Ventana automated immunostainer utilizing paraffin embedded tissue. The strength of staining was graded from 0 to 3+ and pattern of deposition in the tissue was described.

**Results:** The transplant patient group included seventeen patients (10 men; 7 women; age range 32-57 years) who had material available for review from either pancreas transplant alone (5 pts), simultaneous pancreas kidney transplant (5 pts) or pancreas after kidney transplant (7 pts). The control group consisted of eight patients (six women and two men, age range 46-79 years) with pancreas specimens removed for reasons other than related to transplant, namely adenocarcinoma. The studied tissue represented 10 indication (non-protocol) biopsies (two pts with multiple biopsies), 9 explanted allografts and eight pancreaticoduodenectomy specimens. Of the transplant population; four patients (two with multiple biopsies) demonstrated any degree of C4d staining; one pt with 3+/3+ staining had moderate acute rejection progressing rapidly to chronic rejection within 6 weeks over multiple biopsies and was demonstrated to have positive (class 1) donor specific antibodies. Another patient similarly showed rapid progression of vascular disease. The other two patients represented single biopsies with moderate to severe chronic rejection with marked chronic allograft vasculopathy; donor specific antibody status currently unknown. In the control group only one of the eight specimens showed staining which was of 2+ intensity.

**Conclusion:** Our study of C4d staining in pancreas allograft and non-alloraft specimens suggests that this is a specific marker for humoral rejection. C4d was not seen in non-rejection related pathologies in the pancreas allograft group (ischemia, infection, etc.) and was virtually absent in our control population of cases comprised primarily of pancreatic adenocarcinoma. Thus, our study supports the utility of C4d staining in pancreas for the identification of humoral rejection.

### 550 QUANTITATIVE ASSESSMENT OF STEATOSIS IN LIVER BIOPSIES: A PILOT STUDY

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**Background:** The degree of macrosteatosis is an important factor in determining whether or not a donor liver is acceptable for transplantation. Livers with >30% macrosteatosis have a 25% chance of developing graft failure. Currently degree of macrosteatosis is commonly assessed by visual semiquantitive morphologic assessment estimating the percentage of a needle biopsy that is occupied by macrosteatosis. This process is subject to considerable observer variation, particularly on evaluation of frozen section material with the introduction of frozen artefact. Our aim was to assess the utility of stereologic point counting as an objective measure of macrosteatosis using an eyepiece micrometer.

**Design:** A total of 12 archived pre transplant liver biopsies, both with frozen section and corresponding paraffin sections, were randomly selected from the files of the Department of Pathology at Henry Ford Hospital, Detroit, MI. Four representative fields from each biopsy were selected, a point grid lattice (eye piece micrometer) was superimposed and the number of hits on fat globules were counted. Two observers (including one gastrointestinal pathologist) independently scored the specimens. Mean percentage of steatosis in each specimen was calculated. Concordance was assessed using Spearman Correlation Coefficients (SAS v9.12) and mean percentage macrosteatosis between readers was assessed using Wilcoxon Rank Sums Test.

**Results:** Macrosteatosis in the 12 specimens ranged from 0.0% to 16%. Concordance between the 2 readers using Spearman Correlation Coefficients on frozen section slides was r=0.991(p<.0001) and on permanent section slides was r=0.995(p<.0001). The mean percentage macrosteatosis content for frozen versus permanent sections between readers using Wilcoxon Rank Sums Test was not significantly different (p=0.77).

**Conclusion:** Stereologic point counting as an objective measure of macrosteatosis using an eyepiece micrometer is highly reproducible between observers in both frozen and permanent liver biopsies. This approach may have merit as an objective measure of macrosteatosis in the assessment of donor livers for transplantation.

### 551 PATHOPHYSIOLOGICAL CHANGES IN SMALL INTESTINAL MUCOSA OF CHRONIC ALCOHOLIC PANCREATITIS PATIENTS FROM NORTH WEST INDIA

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**Background:** The intake of larger quantities of alcoholic beverages leads to manifold functional disturbances and organ injury in the upper gastrointestinal tract. In many countries alcohol abuse is the most important cause for the development of chronic pancreatitis. Moreover, studies on gastroduodenal morphological changes in patients with this disease and with other alcohol-related conditions have given different results. The pathophysiological change in small intestinal mucosa among chronic alcoholic patients is not known in Indian population. Therefore, this study evaluated the enzyme activities and morphological changes in small intestinal mucosa of patients with chronic alcoholic pancreatitis.

**Design:** In this prospective study duodenal biopsy from diagnosed cases of chronic alcoholic pancreatitis (n = 11) were studied for brush border enzymes (disaccharidases, alkaline phosphatase, LAP, LDH, GGT), intracellular enzymes (G-6-PDH, ICDH, G-6-P), membranous enzyme (Na+ - K+ ATPase) activities and morphological (light microscopic and ultrastructural) changes. The duodenal biopsy from patients with GERD (n = 29) were taken as control. The results were expressed as number and percentage or mean + SE. Comparisons of quantitative measurements between groups were performed with Student's t test. Informed written consent was obtained prior to participation in study. The study was approved by the ethical committee of our Institute.

**Results:** The mean age (all males) of chronic alcoholic pancreatitis patients was 41.0 + 10.7 years. The average consumption and mean duration of alcohol intake was 250 ml/day and 14 + 1.3 years, respectively. In duodenal mucosa of pancreatitis and GERD patients the mean enzymatic activities (Mean + SE IU/g protein) of lactase was 6.2 + 0.5 Vs 24.3 + 1.0 ($p < 0.001$), sucrase was 18.6 + 1.7 Vs 42.4 + 1.8 ($p < 0.001$), maltase was 35.4 + 2.2 Vs 76.1 + 13.0 ($p < 0.001$), LAP was 140.0 + 9.8 Vs 27.7 + 17.6 ($p < 0.01$), LDH was 402.0 + 40.2 Vs 104.9 + 4.1 ($p < 0.001$), GGT was 208.1 + 11.2 Vs 99.5 + 4.2 ($p < 0.001$) and alkaline phosphatase was 267.4 + 11.3 Vs 180.6 + 6.5 KAU/g protein ($p < 0.01$). The mean enzymatic activities in duodenal mucosa of pancreatitis and GERD patients for G-6-PDH was 4.4 + 0.4 Vs 11.0 + 0.2 ($p < 0.001$), ICDH was 13.8 + 0.5 Vs 23.2 + 1.6 ($p < 0.05$), G-