**Results:** Group I -18 cases with abnormal LFT as indicated increased bilirubin, SGOT and SGPT levels. It includes 15 SLE, 2 RA and 1 PAN. Histology- 9 nodular regenerative hyperplasia (NRH); 1 with secondary biliary cirrhosis; 5 with bile ducts fibrosis (BDF), 1 with inflammation; 6 had variable amount of portal tract inflammation (PTI), 5 with inter-phase hepatitis (IH) and lobular inflammation; I had features of PAN; 5 peri-vascular fibrosis (PVF). All cases had fibrosis involving the small sized portal tract (PTF). Group II: 10 cases had normal LFT but 5 cases had mildly raised bilirubin level and included 3 SLE, 2 RA, 2 PAN and 3 SSc. Histology- 6 NRH; 4 BDF; 5 PTI in 1 with IH; 4 PVF; 2 PAN and 9 PTF. Alkaline phosphatase was raised in 11 and 5 cases respectively. Portal vein showed angiomatous transformation in 9 and diffuse fatty changed was seen in 3 cases. There was no correlation between level of enzymes and histological profiles. Five cases with activity and 1 case with necrosis had > 3 folds raised enzyme levels. Wide spectrum of liver pathology was seen irrespective of enzyme levels. All showed one or more than one form of abnormal histology indicating morphological changes in liver in CVD. NRH was seen in more than half of cases.
**Conclusion:** Number of cases with abnormal histology was more than the number of cases with abnormal LFT and alkaline phosphates level. To assess liver status in CVD, a liver biopsy would be indicated in all cases irrespective of the enzyme levels.

### 631 SCREENING OF SERUM AND TISSUE BIOMARKERS IN HEPATOCELLULAR CARCINOMA BY SELDI TECHNIQUE

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**Background:** Liver cancer is one of the diseases with higher incidence and mortality. New technologies for the earlier detection of liver cancer are urgently needed. The aim of this study was to screen serum and tissue biomarkers in patients with hepatocellular carcinoma by using surface-enhanced laser desorption and ionization time-of-flight mass spectrometry (SELDI-TOF-MS) technique.
**Design:** Proteomic spectra were generated by mass spectroscopy in 88 cases, including 34 cases of hepatocellular carcinoma that had been pathologically confirmed with aged 45 to 94 years, and 34 cases of healthy control or liver diseases other than cancer, aged 20 to 78 years. 68 spectra obtained were used to train and develop a decision tree classification algorithm.
**Results:** A total of 17 distinguished proteomic peaks were detected, two of which were used to build a proteomic pattern. The results yielded a sensitivity of 91.18% (32/34), specificity of 97.06% (33/34), and positive predictive value of 96.88%.
**Conclusion:** SELDI-TOF-MS offers a unique platform for the proteomic detection of hepatocelllular carcinoma. It also offers a noninvasive method to further study the proteomic changes in the development and progression of liver cancer.

## Mediastinal

### 632 PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A CLINICOPATHOLOGIC STUDY OF 25 TUNISIAN CASES

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**Background:** Primary mediastinal large B-cell lymphoma (PMBCL) is a subtype of diffuse large B-cell lymphoma with putative thymic B-cell origin. At presentation, it affects mainly the anterio-superior area of the mediastinum. The aim of this study is to analyse clinicopathologic characteristics of 25 patients with PMBCL, diagnosed in our instituation during the last 5 years (from January 2000 to December 2005).
**Design:** This study included 25 patients who presented with PMBCL and for whom formalin fixed embedded tissue was available in the form of mediastinal biopsies (21 cases), computed tomography guided biopsies (2 cases) and sus clavicular lymph node biopsies in 3 patients who presented voluminous antero mediastinal masses. All cases were stained with hematoxylin and eosin to examine morphological features. The elements of histologic examination included tumors cells (centroblastic cells, clear cells, Reed-Sternberg like cells), inflammatory cells, fibrosis, necrosis and angiotropism. For each case, a basic immunohistochemistry panel (CLA, CD20, CD79, CD3, CD5, CD15, CD30, EMA) was used.
**Results:** Median age was 33 years (range 15 to 81 years). 8 patients were male and 17 were female. All patients presented with a respiratory syndrome, 14 with a superior vena cava syndrome and 10 with general symptoms. Chest X-ray revealed widening of the mediastinum and computed tomography scan showed anterior mediastinal mass in all cases. Histologically, fibrosis was present in all cases, inflammatory cells in 20 cases, necrosis in 14 cases and angiotropism in 7 cases. The tumoral cells were exclusively centroblastic in 5 cases, exclusively clear in 3 cases and mixed in 17 cases. Moreover, Reed-Sternberg like cells were observed in 10 cases. Immunostaining showed a positivity for CLA (25 cases), CD20 (24 cases), CD79 (10 cases) and CD30 (2 cases). None of the cases expressed CD3, CD15 or EMA.
**Conclusion:** Primary mediastinal large B-cell lymphoma is a subtype of diffuse large B-cell lymphoma with peculiar features, such as female prevalence, young patient age and bulky presentation. Microscopic features are also peculiar such as fibrosis and pleomophic tumoral B cells (centroblastic cells, clear cells, Reed-Sternberg like cells). Our epidemiological, clinical, radiological and pathological findings are in agreement with published data.

### 633 EXTRAGONADAL MEDIASTINAL GERM CELL TUMORS ARE OFTEN ASSOCIATED WITH KLINEFELTER SYNDROME

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**Background:** Klinefelter Syndrome ( KS) is a well documented abnormality of sex differentiation occurring in 1/600 newborn males. It is characterized by a 47 XXY or a mosaic karyotype and clinical findings include: hypergonadotrophic hypogonadism, small testes, infertility, reduced body hair,gynecomastia and tall stature. Different malignancies such as breast cancer, testicular tumors, leukemia and lymphomas occur in 1-2% of the cases. Patients with KS also have a higher risk for developing malignant mediastinal germ-cell tumors.At least 8% of male patients with primary mediastinal tumors have KS.
**Design:** This report details the molecular cytogenetic studies performed in 4 young males with mediastinal germ cell tumors, none of these cases were previolusly diagnosed as KS To corroborate KS diagnosis, fluorescent in situ hybridization analysis ( FISH) was carried out in paraffin embeded tumor tissue sections in cases 1,3 and 4 , and in peripheral blood in case 2.It was used an X and Y centromeric region labeled with spectrum green and orange respectively. As an internal control an 18 centromeric probe labeled in aqua was used.
**Results:** All 4 cases were young males admitted with severe dyspnea and respiratory distress in which a mediastinal mass was found. In patient 1 the autopsy revealed a mediatinal teratoma with rabdomyosarcomatous transformation confirmed by immunohistochemistry; other autopsy findings were severe tubular atropy, Leydig cell hyperplasia and rete testis pseudohypertrophy which strongly suggested KS. In case 2 a mixed mediastinal teratoma was found ; during physival examination gynecomastia and small testes were observed. He is presently on treatment. In cases 3 and 4 a mature mediastinal teratoma was diagnosed, no other physical abnormalities were found. Both patients died during treatment.FISH analysis performed in cases 1 y 2 revealed a 47 XXY karyotype while the other two were normal XY males.
**Conclusion:** Due to the broad clinical spectrum of KS, some cases lack the full or classical phenotype, and the diagnosis is only made until a complication is present. The association between KS and neoplasms is estimated around 1-2% of the cases but recent data revealed that Klinefelter patients have a relative risk around 66.7 for developing extragonadal germ cell tumors.However, if we include those cases of KS recognized after the diagnosis of the mediastinal germ cell tumor, the incidence is increased and around 20% of all cases of mediastinal germ cell tumors in young males are associated with KS. These data is 50 times more than the expected frequency of mediastinal teratomas.This work supports the association between KS and mediastinal germ cell tumors and insists that in young patients with mediastinal teratoma a cytogenectic analysis must always be performed

### 634 MULTILOCULAR THYMUS CYST WITH ECTOPIC PARATHYROID TISSUE

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**Background:** Thymus cysts are rare. Most of them are probably congenital. The variant which is known as multilocular thymus cyst (MTC) has been considered an acquired lesion of inflammatory origin.
**Design:** The presented case of a MTC associated with ectopic parathyroid tissue is very rare and rises the question of a congenital versus an acquired lesion.
**Results:** A 40-year-old man sought medical attention because of vague thoracic complaints. The radiological examination displayed an anterior mediastinal mass, which showed a minimal growth within a 4 month follow-up. The lesion was resected without complications. The specimen was a 9 x 5 x 1,5 cm mass of firm gray–tan tissue with multiple cysts varying in size from a few mm to 1 cm in diameter. The cysts had a smooth inner surface and some were filled with brown-tan pasty material. Microscopically the lesion was composed of benign thymic tissue with multiple cysts lined with partly keratinizing squamous epithelium that focally showed a continuity with the thymic epithelial structures. At two points there were small foci of ectopic tissue, which were identified as parathyroid tissue due to the histological aspect and the results of immunohistochemistry.
**Conclusions:** The MTC are rare lesions. The association with inflammatory or autoimmune diseases (HIV-infection, Sjögren syndrome, etc.) and tumors rich in inflammatory cells has been repeatedly reported. Consequently, an inflammatory pathogenesis has been postulated, with cytokines of inflammatory cells inducing the cystic transformation of thymic epithelium. Ectopic tissue in a MTC has been mentioned only in very few reports in the literature. Bearing in mind that both the thymus and the parathyroid glands have their origin in the third pharyngeal pouch, the finding of parathyroid tissue in our case of MTC could raise the question of a congenital nature of the MTC in the sense of a malformation or disarrangement. Considering the rarity of this association, the interpretation as a coincidental finding is favoured.

### 635 MUCINOUS ADENOCARCINOMAS OF THE THYMUS: REPORT OF 2 CASES AND REVIEW OF THE LITERATURE

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**Background:** Primary thymic adenocarcinomas are extremely rare neoplasms with only 11 cases reported in the literature; only 3 are of the mucinous subtype.
**Design:** We report two additional cases of the mucinous type, including a previously unreported mucinous variant with numerous psammoma bodies.
**Results:** The first case in a 61-year-old female was a mucinous adenocarcinoma arising from a thymic cyst with areas of transition from benign to dysplastic epithelium. The tumor cells formed dilated glands, cords, and small nests that infiltrated the thymic cyst wall and exhibited evidence of mucin production. Immunohistochemically, the tumor cells were positive for cytokeratin (CK) 7 and focally positive for both CD5 and CK 5/6. The second case in an 82-year-old female resembled a mucinous (colloid) carcinoma of other organs such as the breast and colon. It consisted of islands and strips of tumor cells floating in large pools of extracellular mucin. A unique feature of this tumor was the presence of

numerous psammoma bodies. Immunohistochemically, the tumor cells were positive for CK7 and negative for CD5.
**Conclusion:** The mucinous subtype with psammoma bodies of primary thymic adenocarcinomas should be considered in the differential diagnosis of mediastinal tumors. These two cases provide further documentation of the rare occurrence of primary mucinous adenocarcinomas of the thymic gland.

## Molecular

**636 IMMUNOHISTOCHEMICAL EXPRESSION OF PROTEINS INVOLVED IN BONE METABOLISM: A TMA STUDY OF 74 BONE METASTASIS FROM VARIOUS CARCINOMATOUS ORIGIN**

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**Background:** Carcinoma cells may express proteins involved in bone metabolism, so called osteomimetic properties.
**Design:** In order to assess whether immunohistochemical expression of such proteins is associated with the histologic origin of the primitive tumors or with its metastatic phenotype (osteolytic or osteogenic), we studied immunohistochemical expression of bone sialoprotein (BSP), receptor activator of NFkB ligand (rankl), runx2, bone morphogenetic protein (BMP) 6 and BMP 7 in bone metastatic carcinomas from various origin using tissue micro arrays (TMAs).
**Results:** TMAs were confected from paraffin embedded tissue samples from 74 patients with bone metastasis (BM) originated from : breast carcinomas (14), kidney clear cell carcinomas (13), lung carcinomas (13), prostatic carcinomas (9), colonic carcinomas (6), thyroïd papillary carcinomas (5) and carcinomas from other origins (14) (uterine corpus (3), bladder (2), hepatic (2), gastric (2), uterine cervix (1), epidermoïd (1), unknown (3). Immunohistochemical stainings were evaluated by two independent pathologists. Statistical analyses were made using Chi-2 test and Fischer exact test.
**Results:** BM from breast carcinomas strongly expressed BMP7 (>75%) and moderately expressed other proteins (between 50 to 75 %). BM from kidney clear cell carcinomas moderately expressed BMP6 and weakly expressed (<50%) other proteins. BM from lung carcinomas strongly expressed runx2, while weakly expressed BMP7. BM from prostatic carcinomas weakly expressed BMP6 and strongly expressed other proteins excepted runx2 (moderately). BM from colonic and papillary thyroid carcinomas strongly expressed all proteins excepted runx2 (colonic origin : moderately) and BMP7 (thyroïd origin : moderately). Altogether, a statistically significant difference could only be noted between BMP6 expression by BM from prostatic origin (osteogenic) vs all other origins (osteolytic) (p=6 10-4).
**Conclusions:** Expression of proteins involved in bone metabolism by carcinoma cells, namely acquired osteomimetic properties, is one of the mechanisms supposed to cause osteophilic properties of these tumors. BSP is the main non collagenous bone matrix protein. Rankl is a protein involved in osteoclastic differentiation and activation while BMP6, BMP7 and runx2 are involved in osteoblastic differentiation. The differences observed in immunohistochemical expression of these proteins in the different groups may explain various phenotypes of bone metastasis (osteogenic vs osteolytic). Meanwhile because of limited number of samples, our tests were not strong enough to confirm our hypothesis in most of the cases (runx2 expression between kidney and thyroid BM p=0,029, BMP7 expression between lung an breast BM p=0,04, and between breast and kidney BM p= 0,04, rankl expression between thyroid and kidney BM p=0,029 and between thyroid and breast BM p=0,045)). Our data suggest that carcinoma bone metastasis differ in their osteomimetic properties according to their origin. Predictive and prognostic implications of osteomimetic immunohistochemical profile of such tumors remain to be assessed by larger studies.

**637 HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF E-CADHERIN IN DIFFERENT BREAST LESIONS AND CARCINOMAS**

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**Background:** E-cadherin (EC) is a calcium regulated adhesion molecule expressed in most epithelial tissues.
**Design:** EC was analyzed immunohistochemically on tissue sections of normal, proliferative and malignant breast lesions in an attempt to shed insight on the role of EC. EC expression was assessed semi-quantitatively in four categories: 0 and 1+ were considered negative while 2+ and 3+ were scored as positive.
**Results:** Although 95% of non-malignant proliferative breast lesions showed positive EC immunoreactivity, there was reduced or lost EC expression in all pre-invasive (carcinoma in-situ) breast carcinoma cases. Invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC) cases showed striking differences in their EC expression. None of the ILC cases expressed EC in comparision to a positvity rate of 30% in IDC cases. Further, in the IDC cases, reduced or lost EC expression was associated with high histological grades.
**Conclusion:** There is a significant relationship between EC expression and lobular versus ductal histologic types of breast cancer. Furthermore, reduction or loss of EC expression may be associated with histologic feature of poor differentiation in IDC.

**638 SUB-MEGABASE RESOLUTION TILING (SMRT) ARRAY-BASED-CGH PROFILING AND GENE EXPRESSION DATA PATTERNS IN HODGKIN LYMPHOMA AND ANAPLASTIC LARGE CELL LYMPHOMA CELL LINES**

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**Background:** Hodgkin lymphoma (HL) and Anaplastic Large Cell Lymphoma (ALCL), are forms of malignant lymphoma defined by unique morphologic, immunophenotypic, genotypic, and clinical characteristics, but both overexpress CD30. Identification of molecular markers for prognosis and development of novel targeted therapies depends crucially on a better understanding of HL and ALCL pathogenesis.
**Design:** We determined the characteristic gene expression profile of HL and ALCL and characterized DNA copy number changes in HL and ALCL by Sub-megabase resolution tiling array-based comparative genomic hybridization (SMRT array CGH). HL-derived cell lines (KMH2 and L428) and ALCL-derived cell lines (DEL and SR-786) were analyzed by array CGH and DNA microarray analysis of global gene expression.
**Results:** Significant gene copy number gains and losses were observed on several chromosomes in all the four cell lines investigated in this study. Assessment of copy number alterations with 32433 DNA segments identified an average of 21 genetic alterations. These alterations defined 9 (43%) novel regions not previously reported in the literature. Of the recurrent minimally altered regions (MARs) identified, 12 (57%) were within previously characterized genetic alterations. HL cell lines L428 and KMH2 shared gains in chromosome cytobands 2q22.1-2q24.2, 2q24.2-2q32.1, 9p21-9p12, and 15q26.1-15q26.3, and losses in 4q35.1-4q35.2, 7p14.1-7p21.11, 10q11.21-10q11.23, 13q12.13-13q12.3, and 20p13-20q13.32. ALCL cell lines SR-786 and DEL, showed gains in cytobands 20p13-20q13.32. Additional abnormalities were seen in individual cell lines, but not common to ALCL or HL pairs of cell lines. The use of the gene expression profiling of the same four cell lines used for SMRT array-based CGH, proved to be useful in identifying differentially expressed genes that map to the regions involved in the chromosomal copy number alterations noted in the cells. The minimally altered regions (MARs) 2p16.2-2p13.3, 8q23.3-8q24.12, 9p21-9p12, and 17q21.33-17q24.1 revealed a weak correlation between altered genomic content and gene expression, whereas the other MARs revealed a strong correlation.
**Conclusion:** SMRT array CGH of HL and ALCL cell lines reveals several regions where DNA copy number is commonly gained or lost. Confirmation of these findings could lead to novel therapeutic approaches in HL and NHL. A search for the involved genes located in these chromosomal regions can potentially shed light on the molecular pathogenesis of HL and ALCL. Our study proved that different genes in the same altered region respond very differently to gain and loss of genetic material, probably because of the regulatory mechanism of each gene. This study is considered to be the first one in describing HL and ALCL cell model genomes at sub-megabase tiling resolution. Future studies on the gene and the protein level with primary lymphoma tumor tissue are needed to verify our results and to identify new potential prognostic markers.

**639 HUMAN THYROID CANCER CELL LINES DIFFERENTIALLY EXPRESS PSA, HK6, HK10 AND HK11**

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**Background:** Thyroid cancer (TC) makes up more than 90% of all endocrine cancers. Recurrence and metastasis occur in up to 20% of patients. TC originates mostly from follicular cells. Papillary and follicular well-differentiated carcinomas are the most common histological subtypes. Genetic events underlying PTC include BRAF mutations and chromosomal rearrangements of RET and TRK. RAS mutations and rearrangements of PPARã occur up to 50% of follicular carcinomas. Fine-needle aspiration cytology (FNAC) is the single most informative investigative tool. However, it has limitations and up to 40% of biopsies are either indeterminate or insufficient for conventional microscopic diagnosis. In addition, false-positive and false-negative results occur at intraoperative frozen section analysis. Molecular tools offer an opportunity to improve the diagnostic accuracy of this test by detecting ret/PTC rearrangements and BRAF mutations. Other molecular markers include galectin-3, CD44v6, oncofetal fibronectin, telomerase, high mobility group 1 protein, calcitonin and carcinoembryonic antigen. However, to date, none of these markers, has proven to be adequate to accurately distinguish benign from malignant, or papillary from follicular thyroid carcinoma. We hypothesize that the distinct genetic events involved in the development and progression of thyroid cancer result in the secretion of fingerprint proteins that may allow early cancer detection, and predict tumor behavior and treatment response. Human kallikreins have already been proven to be useful as diagnostic and prognostic markers of several cancer types. In the light of that, we aimed to identify kallikreins expressed in human thyroid cancer cell lines. As a second aim, we assessed the influence of hormonal stimulation in the secretion of differentially expressed kallikreins.
**Design:** TPC-1, MRO and ARO human cell lines, originated from papillary, follicular and anaplastic thyroid carcinoma respectively, were incubated in serum-free medium. After 7 days, the culture medium was collected to measure human kallikreins via ELISA. Additionally, immunohistochemistry was performed in the human cell lines. In a second experiment, cells were grown in medium containing fetal bovine serum and stimulated with hormone aliquots (aldosterone, dexamethazone, estradiol, norgestrel and