ABSTRACTS

PRESENTATION BY TITLE ONLY

T 1 PROFESSIONAL REHABILITATION OF LYMPHOMA PATIENTS : A STUDY OF MEDICAL AND PSYCHOSOCIAL FACTORS ASSOCIATED WITH RETURN TO WORK.

N. Delvaux*, D. Bron*, A. Brédart**, D. Kral*, P. Autier*, L. Debusscher*, P. Stryckmans* and D. Razavi*. *Service de médecine Interne et Laboratoire d'Investigation clinique H. Tagnon. Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, rue Héger-Bordet 1, 1000 Bruxelles, Belgique. **C.A.M., Groupe de Recherche et de Formation. Boulevard de Waterloo, 104 - 1000 Bruxelles, Belgique.

Factors associated with professional rehabilitation of lymphoma patients have been poorly investigated. One hundred and seventeen consecutive Hodgkin's and non Hodgkin's lymphomas patients attending an oncology outpatient clinic were therefore evaluated for their psychosocial status. Psychological distress was assessed by a self administered rating scale, the Hospital Anxiety and Depression Scale (H.A.D.S), treatment toxicity by the W.H.O. grading of acute and subacute toxicity and fatigue by a self administered rating subscale of the Profile of Mood Scale (P.O.M.S). Information were also collected on age, sex, socio-demographic status (Hollingshead Index), return to work (if stopped during treatment), stage of disease and (if out of treatment) time elapsed since end of treatment. Patients retired or housekeepers were excluded from the analysis. Results are showing that among the forty-one patients out of treatment, nineteen (46%) have not resumed professional activities. Univariate analysis shows that resuming job is negatively related to anxiety-depression and treatment toxicity (Wilcoxon rank sum test : p=0.07, p=0.006 respectively) but not related to fatigue, stage of disease, age, sex, Hollingshead Index and time elapsed since end of treatment. The best logistic regression model explaining the association between return to work and the influencing factors combine treatment toxicity, time elapsed since end of treatment, anxiety-depression and a negative interaction between treatment toxicity and time elapsed since end of treatment. This confirms the negative influence of anxiety-depression and treatment toxicity on return to work. The negative interaction indicates that return to work is less probable as treatment toxicity persists over time whereas anxiety-depression has a negative effect independently of time. Long term side effects of treatment and relapses of the disease three years after the first evaluation are currently analysed to look for a relationship between professional rehabilitation and disease-free survival. In conclusion, anxiety and depression as well as physical sequelae due to treatment are hampering professional rehabilitation of lymphoma patients. This indicates the need to implement appropriate psychosocial interventions designed to facilitate professional rehabilitation.

T 2 LYMPHOMA SELF-HELP GROUP AS AN AID TO PATIENT SUPPORT.

T. Hilder, G. Vaughan Hudson. c/o BNLI, University College London Medical School, London, UK.

Non medical support for patients and their families has been increasingly recognised to be of value in supporting the work of clinicians. Since 1986 over 3,500 patients and families have sought help from a national support service in the UK, called the "Hodgkin's Disease Association" (HDA), for those with any lymphoma.

Only Holland, Belgium, Eire and UK have self-help groups specifically for Lymphoma patients and so the HDA has enquiries from across the world. Patients find it important to talk and relate to others specifically with a Lymphoma. The HDA has 800 members and enquiries from patients have increased annually by about 20%, from many sources, to 1,000 a year. The Association was started entirely with volunteers. Important factors in the Association's success are the speed with which enquirers receive basic information coupled with emotional support, strong medical backing, business principles, and an office with proper facilities and volunteer staff now supplemented by paid staff.

Contact with patients and carers is by post, or by a telephone helpline operating 13 hours a day 7 days a week. No personal medical advice is given and patients are encouraged to go back to their doctors for this. Enquirers may be linked by telephone to recommended voluntary "Helpers". A library of videos, books and tapes is much used and a quarterly newsletter is published giving a wider perspective to individual patients, and a sense of community with others. Groups have been formed around the country providing informal social support.

Annual expenditure began at £4,400 and has now risen to £60,000, without allowance for the input of voluntary staff. Patients and their families, who last year provided 58% of the income, are good fundraisers. Funding to supplement this has only actively been sought in the last year from trusts and companies.

To start a support service a core of committed patients with a readily available medical backing is needed. Such an organisation can relieve the time pressures on medical staff, without affecting the doctor patient relationship.

T 3 A PROSPECTIVE STUDY WITH ULTRASONOGRAPHY AND CT IN LYMPHOMA STAGING.

C.Cartoni, AP Anselmo, A.Cafolla *L. Corinto, *E. Pompili, M. Giovannini. Hematology, Dept. of Biopathology; *Institute of Radiology; University "la Sapienza" of Rome, Italy.

Background. Computed tomography (CT) is widely employed for the detection of abdominal disease in lymphomas even though the results are affected by a definite rate of false-negative cases. In order to evaluate if the use of ultrasonography (US) might improve the accuracy of the clinical staging, CT and US were blindly interpreted and agreement between the two imaging test was calculated.
Patients and methods. From November 1991 to January 1993, real-time US was prospectively compared to i.v. bolus dynamic CT in 114 previously untreated patients with lymphoma (59 NHL, 55 HD) in view of abdominal staging. Each operator was unaware of the of the examination performed by the other; time elapsed between the two tests was lesser than 10 days. No histologic gold standard test was used; in the positive cases, follow-up of the imaging abnormalities during the treatment was used to confirm the findings.
Results. US and CT were positive for involvement respectively in 45% and 36% of patients studied. Agreement between US and CT findings was noticed in 82% of patients (kappa = 0.63); US findings were positive and CT findings were negative in 13 patients; the false-negative findings of CT were 10 patients with focal lesions of the spleen and 3 with >1.5 cm lymphadenopaties. In 4 patients with US negative, CT was positive (1 with hepatic focal lesions, 3 with paraortic lymphadenopaties). False-positive findings were observed in 3 patients by using US and in 2 with CT. The combined use of US and CT revealed an infradiaphragmatic involvement in 51 of 114 patients; in 13 patients (11.4%) their disease would not have been detected by using only CT.
Conclusions. The level of agreement between the two imaging techniques was satisfactory. Moreover, in this study US showed a higher sensitivity as compared to CT in detecting splenic involvement. US is therefore recommended to be routinely used in addition to CT for the staging work-up in lymphomas.

T 4 STAGING OF PATIENTS WITH MALIGNANT LYMPHOMA: THE POTENTIAL OF FDG-PET.

MM Henrich, M Bangerter, S Grimmel, N Frickhofen, H Heimpel, SN Reske. University of Ulm, Ulm, Germany

The study was undertaken to assess FDG-PET for diagnosis and treatment control of malignant lymphoma and to compare the results with X-ray computed tomography (CT).

Ten patients (3 with malignant Non-Hodgkin's lymphoma and 7 patients with Hodgkin's disease) were studied with FDG-PET (200-400 MBq) in the fasted state. Tomograms were reconstructed iteratively and visually evaluated. The standardized uptake values (SUV) in the lymph nodes with most intense FDG-uptake and in the vertebral musculature as control region were calculated. Four patients were in stage II, 3 in stage III and IV, respectively.

All patients exhibited intense FDG accumulation in clinically/CT documented manifestations. Mean SUV was in the range of 2.1 to 25.4 (n=10) for lymphoma, the soft tissue contrast ratio was in the range of 3.5 to 38.2. In 6/10 cases PET demonstrated additional nodal manifestations. In 3 patients with stage IV disease, lymph nodes in axillar, supra-clavicular, mediastinal , paraaortal or inguinal localization, respectively were detectable clearly and with high contrast. In one patient residual viable affected lymph nodes were detected with PET 3 months prior to other imaging modalities.

Thus FDG-PET revealed intense FDG-accumulation in all known manifestations of malignant lymphoma. Additional affected nodes were a common finding. Whole body FDG-PET has the potential to provide accurate staging and treatment control.

T 5 CLINICAL APPLICATIONS OF FDG-PET IMAGING IN MANAGEMENT OF MALIGNANT LYMPHOMA

H.G.Bischoff°, M.V.Knopp*, M.Bucher°, P.Drings°, G.van Kaick*

°Thoraxklinik Rohrbach, Dept. of Medical Oncology, Heidelberg ,FRG
*Radiological Dept, German Cancer Research Center, Heidelberg

We evaluated the potential of clinical application of fluordeoxyglucose (FDG) PET imaging in the initial staging of malignant lymphoma, for therapy monitoring, and for detection of tumor recurrence.
Twelve patients with histologically confirmed malignant lymphoma were imaged with PET. Seven patients were initially studied prior to therapy. Five were studied for recurence. Ten follow-up studies have already been performed. A previously established optimized imaging protocol for body PET imaging was used. The PET images were evaluated visually as well as quantitatively.
All malignant lymphomas showed high FDG uptake and were clearly identified. Successfull therapy resulted in rapid decrease of FDG uptake. The assessment of bulky scar tissue compared with active residual tumor proved to be very helpfull (n=5). All classifications based on PET agreed with the histological findings when obtainable (n=7). The remaining findings agreed with the results of the clinical follow-up.
We can conclude that FDG-PET imaging shows great potential for clinical applications in the asessment of malignant lymphoma, especially in patients with residual mediastinal mass after therapy.

T 6 ECHOCARDIOGRAPHIC DIAGNOSIS OF RIGHT VENTRICULAR OUTFLOW TRACT OBSTRUCTION DUE TO EXTRINSIC COMPRESSION BY LARGE CELL LYMPHOMA.

S. Gillis, E. Dan, C. Putterman, D. Gilon, A. Polliack.
Leukemia/Lymphoma Unit, Department of Hematology, Division of Medicine, Department of Cardiology, Hadassah University Hospital, Jerusalem, Israel.

Acquired right ventricular outflow tract obstruction due to extrinsic compression of the pulmonary artery is a rare manifestation of non-Hodgkin's lymphoma (NHL). Recently we encountered a case of a 17 year old boy who was referred for evaluation of a large anterior mediastinal mass, causing dyspnea and cough and resulting in a harsh systolic murmur. Echocardiography demonstrated compression of the pulmonary artery by the mass, with a severe pressure gradient. Biopsy revealed intermediate grade, diffuse large cell NHL. Systemic chemotherapy rapidly led a to a significant decrease in the size of the mass, and virtual disappearance of the pressure gradient. In this report, the use of echocardiography for diagnosis and follow up of extracardiac tumors is reviewed. It is suggested that this technique may be useful for the routine staging of mediastinal lymphomas because of the potential consequences of clinically undetectable hemodynamic compromise.

T 7 Evaluation of Residual Mediastinal Mass in Malignant Lymphoma. A Pilot study evaluating Computed Tomography (CT), Magnetic Resonance (MR) and 67 Galium Scanning (Ga-scan).

A.F.Abrahamsen, H.Lien, M.Aas, B.Hager, M.Lindegaard, O.Nome, S.Kvaløy.
Dept. Oncology, Radiology and Nuclear Medicine, Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway

Most malignant lymphoma patients with a mediastinal bulky tumor have a residual mediastinal mass at the end of treatment. To determine if this mass represents active disease or not, CT,MR and Ga scan were performed before treatment, after chemotherapy, after radiotherapy and 6 months post-therapy in 19 lymphoma patients. Thereafter, CT was done at least twice each year. The mean observation time after treatment was 32 months (24-39 months).

In 17 patients, MR and Ga scan showed the same result. Both MR and Ga scan were negative in 11 patients with residual mass, and none of these patients have shown an increase of mediastinal mass, although 3 of these patients have had a relapse below diaphragm. 3 patients who had CT,MR, and Ga scan indicating residual tumor. These patients had regrowth in mediastinum within 1 year. 3 patients had negative CT, MR, and Ga scan, and none of these had relapse.

In only two patients the results of MR and Ga scan differed. One patient with residual mass who had a negative Ga scan and a postive MR had a relapse; but another patient with a positive CT, negative MR, and positive Ga scan is relapse free.

It is concluded that a residual mass is found in most patients after a bulky tumor. Both MR and Ga scan are valuable for separating rest tumor from indolent residual mass.

T 8 *HYPERCALCEMIA IN HEMATOLOGIC MALIGNANCIES: A PROSPECTIVE STUDY OF THE CLINICAL ASSOCIATIONS AND THE ROLES OF HUMORAL MEDIATORS.*

JF Seymour, FB Hagemeister, F Cabanillas. U.T. M.D. Anderson Cancer Center, Houston, Texas 77030.

Parathyroid hormone-related protein (PTHrP) is the major mediator of humoral hypercalcemia (HC) in solid tumors and may be involved in HC complicating hematologic malignancies. 1,25-dihydroxyvitamin D_3 (Vit D) can also cause HC in Hodgkin's disease (HD) and non-Hodgkin's lymphomas (NHL). The incidence of these mechanisms of HC is unknown and the simultaneous involvement of both mediators has not been studied.

HC was defined as a corrected calcium (CCa) [total calcium + 0.8(4.0 - albumin)] of ≥11.0 mg/dl. From July to December 1992, 34 consecutive patients (pts) with HC were identified and 30 studied within 48 hours of the diagnosis of HC and prior to chemotherapy. 3 pts (10%) had elevated levels of intact PTH, signifying co-existent primary hyperparathyroidism. Of the remaining 27 pts, 11 (41%) had multiple myeloma (MM), 1 (4%) HD, and 15 (56%) NHL (low grade in 6, intermediate/high grade in 9 by the International Working Formulation). 2 pts had T-cell phenotype, and 1 pt was HTLV-1-antibody seropositive. Results of blood tests are shown, expressed as median values:

	CCa	PO_4	Creat	PTH	PTHrP	Vit D
Units	mg/dl	mg/dl	mg/dl	pg/ml	pmol/l	pg/ml
Normal range	8.4-10.2	2.5-4.5	0.8-1.5	11-54	<1.5	20-76
MM (n=11)	12.2	4.0	1.8	8	<0.2	5
p value	>0.1	>0.1	>0.05	>0.1	>0.1	0.0025
HD/NHL (n=16)	11.8	3.5	1.2	10	<0.2	24

Pts with MM and HD/NHL were of similar ages, sex distribution, and albumin (p>0.1). In 5 HD/NHL pts the probable cause of the HC was identified; 1 HD and 3 int./high grade B-cell NHL pts had elevated Vit D levels, and 1 pt with lymphocytic lymphoma/CLL had a minor elevation of PTHrP (measured by specific two-site immunoradiometric assay) at 1.8 pmol/l. There were no differences between pts with and without elevated Vit D levels (p>0.05). Pts with int./high grade NHL had a higher CCa than pts with low grade NHL (medians 12.4 and 11.0, respectively), p=0.05. These results 1/ confirm the suppression of Vit D and fail to confirm any systemic action of PTHrP in MM, 2/ demonstrate that Vit D elevation in B-cell NHL is not due to the action of PTHrP on renal Vit D metabolism, and 3/ suggest that Vit D is a common cause of HC in this patient group, accounting for 20% of cases of HC in NHL.

T 9 DIABETES INSIPIDUS AS CIRCUMSTANCE OF DIAGNOSIS OF NON-HODGKIN LYMPHOMA (NHL).

R. Bouabdallah, JR Harle*, J. Camerlo, S. Molinier, T. Aurran, N. Horchowski*, PJ. Weiller*, JA. Gastaut. Institut J. Paoli-I. Calmettes, Marseille, France *Hopital La Timone, Marseille, France.

We report the case of a man, 50 years old, who was admitted in our institution on August 1992 with a diagnosis of NHL. On June 92, Mr. GI... underwent surgery for compression of the right nervous ulnaris. A few days later, this patient presented a diabetes insipidus. He efficiently responded to DESMOPRESSIN treatment. In the same time, appeared lumbago and paraparesis. This patient was then admitted in neurosurgery department, where laminectomy of the second lumbar vertebra was performed. Histological diagnosis of the bone tissue concluded to a NHL of intermediate grade of malignancy. In our Institution, a complete staging was done and concluded to a stage IV (bone) NHL. We parallely investigate hypophysis of this patient in order to etablish it's specific involvement. Before chemotherapeutic treatment of NHL, hormonal titrations confirmed a hypophysis deficiency (see table). The CTscan shows thickness of pituitary infundibulum (8 mm). MRI of hypophysis shows the same abnormality on the pituitary infundibulum. A visual field was equally performed, showing scotoma on the left eye. The patient recieved chemotherapy (CT) regimen and was evaluated for hypophysis function after three courses. Need of DESMOPRESSIN was less frequent since the first course. Hormonal titrations increased and reached normal values (see table). A new MRI showed normalization of pituitary infundibulum. At the same time, NHL was considered in complete remission. In this original report, diabetes insipidus was the circumstance of diagnosis of NHL.
Such a localisation in NHL is rare. Even no histological diagnosis was assessed on hypophysis, correction of hypophysis function after CT is an argument of lymphomatous infiltration of the gland.

	TSH (mUI/l)	FT3 (pmol/l)	FT4 (pmol/l)	FSH (UI/l)	LH (UI/l)	ACTH* (pg/ml)	CORTISOL* (ng/ml)
Normal values	0.4 - 4	3 -10	8 - 21	1.5 - 8	1.5 - 7	20 - 55	200 - 700
Before CT	2.1	3.3	8.9	< 1	0.2	5	30
After CT	1.4	5	10.4	9.2	1.1	13	NE

* Values at 8.00 a.m.
NE = not evaluated

T 10 LOCALIZED HIGH GRADE B-CELL LYMPHOMA OF BONE FOLLOWING CHRONIC OSTEOMYELITIS EXPRESSES AN ADHESION RECEPTOR PROFILE CORRESPONDING TO A NON-DISSEMINATING PHENOTYPE. *A case report.*

M. Schmitt, H. Goldschmidt, and P. Möller
Dept. Internal Medicine, Division of Hematology, and Institute of Pathology, University of Heidelberg, Heidelberg, Germany

A 56 years old male with a 30 year history of chronic osteomyelitis of his right femur was admitted with a spontaneous fracture of this bone after two years of pain in his right limb. Surgery revealed a bulky tumor including great parts of the femur.

Histology showed a high grade malignant B-cell lymphoma destroying the bone. The lymphoma cells were described as atypical, centrocyte-like and medium-sized to large. Proliferation rate (Ki-67) was 60 %.

The tumor cells expressed the following immunophenotype: IgM-; IgD-; IgG-; IgA-; kappa and lambda light chains-; CD10-; CD20+; CD22+; CD30-; CD38-; CD39-. This immunoprofile is indicative for a B cell differentiation other than the follicular center and the plasma cell stage of development. It might correspond to the extrafollicular stage of differentiation.

It has repeatedly been demonstrated that extranodal lymphomas arise in sites of chronic inflammation (e.g., B-cell lymphomas of the salivary gland in Sjögren's syndrome or B-lymphoma of the thyroid after Hashimoto's thyroiditis; gastric lymphoma in chronic lymphofollicular gastritis, etc.). These lymphomas tend to stay localized for a considerable time. This might be due to the adhesion receptors on the tumor cells.

The adhesion receptor profile of this lymphoma was as follows: β1-integrins (VLA) -; β7-integrin β-chain-; LFA-1 (CD11a/CD18)+; CD11b-; CD11c-; ICAM-1 (CD54) weakly +; LFA-3 (CD58) weakly +; CD44+, LECAM-1-.

Taken all available data of the current literature together, the a-leukemic / locally growing phenotype of a B-cell lymphoma might be determined as VLAs-; LFA-1+; ICAM-1+; CD44- and LECAM-1-. Thus, this tumor which did up to present not show any signs of dissemination, corresponded in 4 aspects to this pattern.

T 11 RARE CASE OF MIXED GERMINOGENIC BLASTOMA AND HODGKIN'S DISEASE OF THE OVARY. MORPHOLOGICAL AND CYTOGENETICAL ANALYSIS.

G.Ganchev, K.Nikova I.Atanassova, G.Chavracov. National Oncological Center, Sofia 1156, Bulgaria

G.B.G. 16 years old, schoolgirl with slowly progressing body hair virilization..Since 2-3 years a significant hoarseness of the voice occured. The histological and elektronmicroscopical analysis reveald a mixed germinoge nic blastoma of both gonads including malignant androblastoma, teratocarcinoma and Hodgkin's disease/ lymphocytic and histiocytic predominance/. The chromosome study was done on peripheral blood leucocyte cultures. In our case we found 46 xy kariotype with a translocation 1:9/ p2.22 - q3.32/ on stage 600-900 band. The mother and broter of the proband are phenotypically normal although remains open the question of the association ofoncogene in chromosome 9 long arm with the gonadal malignation in our patient.

T 12 PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION IN CHILDHOOD LYMPOMAS.

M. Kalmanti, P. Kanavaros, A. Sakalidou, M. Tzardi, E. Kazlaris. Department of Pediatric Hematology-Oncology and Pathology, University Hospital of Heraklion, University of Crete, Greece.

Paraffin sections from 21 cases of Hodgkin's disease (HD) and 28 cases (26 high grade and 2 low grade) of Non-Hodgkin's lymphomas (NHL) occuring in childhood were examined for the presence of proliferating cell nuclear antigen using an anti-PCNA antibody. All cases of HD and NHL showed PCNA reactivity. In HD 50,9 % (mean value) of Hodgkin and Reed-Sternberg (HRS) cells were PCNA positive and judged to be proliferating PCNA reactivity was also found in a variable number of cells of the background population in HD. In NHL 61,2% (mean value) of cells were PCNA positive. In the 26 high grade tumors 69,6% (mean value) of cells were PCNA positive while only 32% (mean value) of cells were PCNA positive in the 2 low grade tumors. Our results show that the proliferation rate of tumor cells in high grade NHL Is higher than those of tumor cells in low grade NHL and HRS cells in HD. Moreover, we found a considerable variation of proliferation rate among individual cases of HD (range 31%-68%) or NHL (range 31%-78%). This suggests that PCNA assessment can help in the individual approach of the proliferation rate of each tumor, a parameter of potentially importance for predicting the biological behaviour of the tumor and the prognosis of the disease.

T 13 EXPRESSION OF THE RECEPTOR FOR THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR IN NORMAL AND NEOPLASTIC HAEMATOPOIETIC CELLS.

E. Ralfkiaer, T. Plesner, M. Wittrup, H. Johnsen, C. Pyke, N.E. Hansen, K. Danø. Departments of Pathology and Haematology, Herlev Hospital, University of Copenhagen, Denmark; Department of Tumour Cell Biology and Pathology, Rigshospitalet, University of Copenhagen, Denmark; and Department of Pathology, Gentofte Hospital, University of Copenhagen, Denmark.

Expression of the receptor for the urokinase type plasminogen activator (uPAR) has been studied by flow cytometry and immunohistology in normal blood and bone marrow cells, in vitro activated lymphoid cells and tissue samples from reactive lymph nodes (n=5) and malignant lymphomas (n=80) or leukemias (n=27). HL-60 myeloid precursor cells and CD34-positive normal stem cells were also analysed. In the normal cells, staining was confined to monocytes, macrophages, neutrophils and myeloid precursors. No labelling was seen of normal or activated lymphoid cells. Purified CD34-positive haematopoietic progenitors were uPAR-negative, but expressed uPAR during differentiation in short term liquid culture stimulated by IL-1, IL-3, IL-6, GM-CSF, and G-CSF. Enhanced uPAR expression was also seen in HL-60 cells after induction of differentiation with DMSO or etalpha. In lymphomas and leukemias, the staining pattern was similar to that seen in the normal cells with labelling of monocytic and myeloid malignancies and no staining of B-cell or T-cell lymphomas or Hodgkin's disease. It is concluded that uPAR is a differentiation marker for myeloid and monocytic cells and may act to facilitate migration of these cells in normal and pathological conditions. The data also indicate that uPAR is useful for the recognition of myeloid and monocytic malignancies. Whether the expression of uPAR in these conditions relates to their growth and behaviour will be an important topic for investigations in the future.

T 14 CLINICAL INVESTIGATION FOR THE CHEMOTHERAPY TO MALIGNANT LYMPHOMA PATIENTS WITH HEPATITIS VIRUS

F. Kodama, T. Noguchi, K. Ogawa, A. Maruta et al. Division of Hematology/Chemotherapy, Kanagawa Cancer Center, 241 Yokohama, Japan

We tried a clinical investigation to seek a strategy for the effective chemotherapies which do not induce severe hepatic damage to malignant lymphoma(ML) patients carrying hepatitis virus. We had ten HBS-Ag positive patients (male/female:9/1, median age:53, CS II:2, III:4, IV:4) out of 165 consecutive ML(including IBL) patients from Jan. 1984 to Dec. 1992. All the ten patients were treated with any chemotherapy regimens including adriamicin. As for the liver condition before the chemotherapy, they were consisted of 8 asymptomatic, 1 symptomatic and one liver cirrhosis patients. After the chemotherapy, 3 patients stayed asymptomatic or had no exaggeration in the liver function, and 5 exaggerated, and 2 became fulminant hepatic failure(FHF) which resulted in death. In most of the exaggerated or FHF cases, exaggeration or FHF in the liver dysfunction appeared during the 2nd or 3rd or the later chemotherapy cycles and during the recovering periods from bone marrow suppression with a preceding increase of HBV DNA polymerase. WBC nadir after the chemotherapies didn't correlate with the liver damages. Two patients with the HBS-Ag titers(EIA assay) less than 64 before therapy didn't have any or further liver damage, but 6 patients with the higher titer than 64 showed exaggerated liver function or FHF. We used IFN-β with or without prednisolone or cyclosporine A to prevent severe hepatic damages for the latest patients and acquired the successful results.

T 15 INFECTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA. ANALYSIS OF INCIDENCE AS A FUNCTION OF THE LENGHT OF FOLLOW-UP.

S. Molica. Divisione di Ematologia,Ospedale Regionale "A. Pugliese",88100 Catanzaro,Italy.

Infections represent the major cause of death in chronic lymphocytic leukemia (CLL),however,clinical studies dealing with their incidence have yielded inconclusive results. In order to address such an issue we reviewed the records of 125 CLL patients (mean age,65.6 yrs;81 M/44 F;Stage A,48;Stage B,37;Stage C,40) followed-up at our institution over a 10-year period. The 125 patients accrued 447 person-years,a mean of 3.8 years per person. There were 119 recorded infections: 47 severe (crude rate 9.8 per 100 person-years) and 72 moderate,respectively. The 5-year risk of developing a severe infection for the whole series was 26% (95% CI:24.7-27.3%) and 20 out of 71 deaths (28.1%) could be attributed to infectious causes. Despite a linear trend toward an increased risk (r=0.98) the hazard function analysis showed a constant pattern of risk (r=0.30),suggesting a lack of correlation of such an event with time. Furthermore,the 5-year risk of developing a severe infection increased to 57.1% (95% CI:36.4-77.8%) for patients with low IgG levels (less than 6.5 gr/dl) and to 68% for those with both low IgG levels and disease stage C. On the other hand,patients who experienced a severe infection had more frequently advanced clinical stage ($P<0.001$),low IgG levels ($P<0.001$) and diffuse bone marrow (BM) histology ($P<0.05$)(Tab 1).
Infection is a constant risk in CLL that is associated with shortened survival. Factors such as hypogammaglobulinemia and advanced disease appear to be the major predisposing factors.

Table 1. PATIENT CHARACTERISTICS

	No infect.	Moderate inf.	Severe inf.	P
N. pts	63	30	32	
Sex (M/F)	41/22	20/10	20/12	NS
Age (yrs)	65 ± 8.2	69.5 ± 7.5	62.6 ± 9.5	NS
Follow-up (mo.)	51.4 ± 37	47.1 ± 29.3	40.4 ± 32	NS
Stage (A/B/C)	33/17/13	15/9/6	0/11/21	<.001
IgG (<6.5 gr/dl)	15.8%	23.3%	56.2%	<.001
BM (D versus non-D)	20/5	3/5	4/5	<.05

T 16 NON-HODGKIN'S LYMPHOMA WITH EXTRA-NODAL INVOLVEMENT : PRESENTING FEATURES AND FINE NEEDLE ASPIRATION CYTOLOGY DIAGNOSIS.

D.K. Das, S.K. Gupta , Postgraduate Institute of Medical Education & Research, Chandigarh, India ; Institute of Cytology & Preventive Oncology, New Delhi, India and Faculty of Medicine, Kuwait University, Kuwait.

339 cases of non-Hodgkin's lymphoma were diagnosed by fine needle aspiration (FNA) cytology over a period of 19 years (1974-1992). 128 cases (37.8%) had extra nodal involvement. The age of these 128 patients ranged from 2 to 82 years with two peaks in 3rd and 6th decades. Male to female ratio was 89 : 39. Clinical diagnosis was lymphoma in 56 cases and lymphoma was one of the possibilities in 16 cases. There were extra-nodal tumors alone in 54 cases and in 74 there was lymph node involvement along with extra nodal tumors. Common extra nodal sites were gastrointestinal tract (32 cases), bony lesions (32 sites in 26 cases), subcutaneous nodules (17 cases), soft tissues (16 cases), liver (12 cases) and salivary glands (11 cases). Sites of FNA were extra nodal sites alone in 69 cases, lymph nodes alone in 42 and extra-nodal sites as well as lymph nodes in 17 cases. Cytodiagnosis was low-grade lymphoma in 9 cases (2 small cell, CLL, 2 small cell, lympho plasma cytoid and 5 small cell, cleaved), intermediate-grade lymphoma in 16 cases (4 mixed small & large cell ; 5 large cell, cleaved and 7 large cell, non cleaved) and high-grade lymphoma in 95 cases (23 large cell, immunoblastic ; 6 lymphoblastic, non convoluted cell type ; 12 lymphoblastic, convoluted cell type ; 40 small non cleaved, Burkitt-type and 14 small non cleaved, non-Burkitt -type). Remaining 8 cases were miscellaneous type lymphomas. Frequency of extra-nodal involvement in low, intermediate and high-grade lymphoma was found to be 23.7%, 21.9% and 44.6% respectively. Small non-cleaved Burkitt-type lymphoma was most frequently (80.0%) associated with extra-nodal tumors. Histopathology was done in 60 (46.9%) cases. There was cyto-histology agreement for diagnosis of NHL in 59 (98.3%) and for specific sub-type of NHL in 43 (71.6%).

T 17 USE OF FINE NEEDLE ASPIRATION OF LYMPH NODES FOR DIAGNOSIS AND FOLLOW-UP IN LYMPHOMATOUS PROLIFERATIVE DISORDERS.

A. Berrebi, M. Shtalrid, E. Vorst, L. Bassous- Guedj. Hematology Institute, Kaplan Hospital, Rehovot, Israel

Percutaneous fine needle aspiration (FNA) is a rapid, minimally invasive technique for cytologic evaluation of lymphadenopathy and tumors. Non Hodgkin's lymphoma (NHL) can often be diagnosed by FNA cytology, but that is not sufficient for subclassifying the disease. During the last 5 years there is a renewed interest in this technique. Several reports deal with the accuracy of FNA compared to surgical biopsy. It seems that the challenge is not only to make a rapid diagnosis but rather a first step to segregate malignant from non-malignant conditions, to evaluate relapses and to get easily cells to be used for modern techniques (immunophenotyping, immunocyto- chemistry, cytogenetics, DNA studies). This allows a more accurate diagnosis than cytology alone. During the last seven years we performed routinely FNA in patients presenting lymphadenopathy or extranodal tumor mass, both de novo and/or during follow-up. The patients were referred by the departments of otolaryngology, clinical oncology, hematology and pediatrics. Air-dried slides were stained by May Grunwald-Giemsa. 687 procedures were performed and reported as not diagnostic (98), normal lymphoid or other tissue - thyroid salivary gland, cysts (123), non-malignant - purulent, reactive, lipoma (201), metastasis (120), lymphoma (103), Hodgkin's disease (22) and suspected lymphoid disorder (20). The accuracy of the FNA in hodgkin's disease was 90%; one case was reported as probably lymphoma but on biopsy the very unusual picture of lymphocytic predominance nodular HD was made. Another case with numerous histiocytes suggesting Reed Sternberg cells was toxoplasmosis. In NHL, FNA was performed as an initial procedure in 51 cases, confirmed by biopsy in 48 and by clinical course in 3. During follow up FNA was performed in 52 cases, biopsy confirmed relapse in 21, while in 31 the clinical course was compatible with relapse. Twenty cases were reported suspected for lymphoma, 3 were proved to be lymphoma at biopsy; the remaining cases were reactive including lymphadenopathy, Kikushi's, toxoplasmos, sarcoidosis, TB. In 5 additional cases,reported as anaplastic or metastatic, the true diagnosis was large cell lymphoma. Adequate cytology permitted to identify the subtypes of lymphoma as follows: mixed small and large (11 cases), large immunoblastic (15), Burkitt (5), well differentiated with plasmocytoid differentiation (3). In 6 cases that were not candidates for surgical biopsy, we used cells for immunotyping and could diagnose B cell lymphoma in 4, Burkitt's lymphoma in 1 and normal phenotype in 2. The results demonstrate the usefulness of FNA in diagnosis and management of lymphoma.

T 18 VERAPAMIL INHIBITS B CELL PROLIFERATION AND TUMOR NECROSIS FACTOR RELEASE AND INDUCES A CLINICAL RESPONSE IN B CELL CHRONIC LYMPHOCYTIC LEUKEMIA.

A. Berrebi, M. Shtalrid, A. Klepfish, L. Bassous-Guedj, M. Kushnir, L. Shulman, and T. Hahn. Kaplan Hospital and Weizmann Institute of Science, Rehovot, Israel

Verapamil is a calcium channel blocking agent which potentially reverses multiple drug resistance in myeloma. So far, no role for verapamil has been reported in management of other lymphoid malignancies. In four B CLL patients, treated with verapamil for cardiac problems, we observed a substantial reduction of lymphadenopathy in one, a 3 and 5 year stabilization of B CLL in two patients and a dramatic decrease in lymphocyte count, lymphadenopathy and splenomegaly in one stage IV patient. We studied the effects of verapamil on B CLL cells in vitro. Peripheral blood mononuclear cells (PBMN) at 10^6/ml, from 10 B CLL patients were stimulated for 5 days with pokeweed mitogen (PMW, 10 μg/ml), PMW + verapamil (40 μg/ml) and verapamil alone. Proliferation was assessed by measuring uptake of [^{3}H]TdR. In all cases we observed that verapamil strongly inhibited in vitro proliferation of PMW-stimulated and unstimulated cells. Since intracellular free Ca^{++} flux is the initial event in B cell proliferation, we measured it by flow cytometry in Fluo3 AM- loaded B CLL cells stimulated by anti-μ in the presence or absence of verapamil. Preliminary results showed that about 10% of the anti-μ-stimulated B cells incorporated calcium in contrast to 0% when verapamil was added. We also examined the release of tumor necrosis factor (TNF), an autocrine growth factor for B CLL cells. Using a cytotoxic bioassay we observed that verapamil markedly inhibited the spontaneous and PWM-induced release of TNF by PBMN cells. These cells constitutively secreted large amounts of IL6 which was not increased by treatment with PWM and was unaffected by verapamil in vitro. These findings suggest that verapamil may block B CLL cell proliferation through inhibition of TNF release and thereby may contribute to the management of B CLL.

T 19 FEASIBILITY OF INFUSION VERAPAMIL ASSOCIATED TO EPOCH CHEMOTHERAPY IN THE TREATMENT OF DRUG RESISTANT LYMPHOMAS.

P. Tagliaferri, P. Correale, M. Mottola*, R. Ascione*, A. Morabito, A. Rea, M. Caraglia, E. Matano, V. Montesarchio, R. Lauria, G. Palmieri, A.R. Bianco.
Cattedra di Oncologia Medica e di Cardiochirurgia*, Facoltà di Medicina e Chirurgia, Università Federico II Napoli.

Several clinical trials which associate Verapamil administration to cytotoxic chemotherapy have been designed for circumvention of drug resistance. We have evaluated cardiovascular side effects produced by VP and EPOCH chemotherapy in 8 patients with chemoresistant lymphomas. VP has been administered as 5 days contnuous infusion at 0.2 mg/kg/h after 0.15 mg/kg bolus injection, with progressive dose reduction on days 6, 7 and 8. EPOCH chemotherapy consisted of 5 days continuous infusion: Etoposide (50mg/m2/day), Doxorubicin (10 mg/m2/day), Vincristine (0.4 mg/m2/day); Cyclophosphamide was administered as 750 mg/m2 bolus injection on day 6 and Prednisone 60 mg/m2 on days 1-14. Continuous monitoring of right intracardiac pressure, ejection fraction and cardiac index has been performed in three patients through Swan-Ganz cathether. While slight reduction of ejection fraction could be detected 6h after beginning of VP infusion, cardiac function was not depressed in all patients. Grade 1 AV block was detected in 5/8 pts, while all patients experienced mild and transient hypothension. Hypokaliemia was detected in 6 pts, which could not be ascribed to gastrointestinal or renal loss. Premature ventricular beats occurred in 1 pt, with prompt recovery. We observed 4 complete responses and 1 partial response. We conclude that EPOCH + VP appear to show in our hands a clear antitumor activity that needs confirmation in larger series, moreover it appeared well tolerated and side effects could be easily managed.

T 20 CLINICAL AND MORPHOLOGICAL STUDY OF LIVER AND BONE MARROW LYMPHOCYTIC INFILTRATION IN ESSENTIAL MIXED CRYOGLOBULINEMIAS.

A. Monteverde, G. Airoldi, P. Zigrossi, G. Bordin, G. Angeli*, S. Pileri**, M. Ballarè. General Medicine II and (*) Pathology Dept., Ospedale di Novara; (**) Pathology Dept, Policlinico S.Orsola, Bologna, Italy.

Essential mixed cryoglobulinemias (EMC) are considered as autonomous entities for their omogeneous clinical manifestations. On the contrary, we consider EMC as symptoms of other diseases, in which polyclonal (EMC type III) or monoclonal (EMC type II) B lymphoproliferation causes the appearance of the cryoprecipitable IgM.
To verify this hypothesis, we have reviewed 70 consecutive cases of EMC II observed in our center since 1985, in whom multiple liver and bone marrow biopsies were performed during the follow-up (in total, 94 liver and 131 bone marrow biopsies).
At liver biopsies, in 40/70 patients we found portal infiltrates without piecemeal necrosis, with pictures resembling chronic persistent hepatitis; in 31/40 a prevalence of CD22+ B lymphocytes (IgMκ in 30, IgMλ in one) was found at immunohisochemistry. This picture was prevalent in early stages of the disease, evolving in the long term to chronic active hepatitis and/or cirrhosis with prevalence of T lymphocytes.
Similarly, 67/70 bone marrow biopsies were characterized by paratrabecular, nodular lymphoyd infiltations, not centered on blood vessels, larger than 200 μm, with morphological and immunochemical features identical with those of the liver, with pictures resembling the lymphoplasmacytoid immunocytoma of the Kiel classification.
These data support the hypothesis that EMC could be an expression of a lymphoma-like lymphoproliferation, mainly located in liver and bone marrow, secreting IgMκ; in this sense, similarities can be found between EMC and MGUS or cold agglutinin diseases.
Recent observations (and our experience) have emphasized the role of hepatitis C virus (HCV) in the pathogenesis of EMC through a virus-induced lymphoproliferation of B cells, possibly CD5+: indeed, HCV can infect the B lymphocytes, and often causes the appearance of nodular infiltrates (rich in polyclonal B cells) in chronic hepatitis C.

T 21 SECONDARY REARRANGEMENT IN MURINE T CELLS CLONES : A MODEL OF LYMPHOMATOGENESIS

by JP Marolleau, P A Cazenave, K Marcu and D Primi

Unite d'immunochimie Analytique - Institut Pasteur 25 rue du Dr Roux - 75724 Paris France.

Few experiments on murine T cell lymphoma have been described. In order to obtain immortalized T cell clones , we inoculated newborn mice with recombinant ecotropic retrovirus harboring c-myc oncogene alone or in combination with H-ras oncogene (Eur J Immuno 1988 18 1101-1109).After few weeks Lymphoma Tumors from spleen ,thymus or lymph-nodes were present. T cells clones have been obtained from theses lymphomas tumors by limiting dilution on feeder cells . They were inoculated in BALB/c or C.B20 mice recipients .Different subclones were generated from secondary tumors .The rearrangements of TcRß and α chains genes and heavy chain gene of immunoglobulin were analyzed by Southern Blot . Tcells subclones obtained from M14 T,M31T,M8T and M15S have undergone secondary rearrangements.All theses subclones display the same TcR γ chain rearrangements as the parental lines.In subclones from M14T (Cell Vol 55 291-300 1988) and M31T we detected new V α _ J α rearrangements on one or both alleles .For M31T subclones secondary rearrangements occurred on the two alleles with preferential utilisation of V α A10 in 3 subclones in association with different J α .In one CD3 negative M8T subclone a deletion of one allele of TcR ß chain occured . We were surprised to detect a new Dh-Jh rearangement in one M15S subclone without change on TcR genes .
In summary new TcR or IgH rearrangements could occur in differents murines Tcell clones after in vivo passages . Oligoclonality ,Vh or Vκ replacements have been described in murine or human B cell lymphoma (Levy et al). These data demonstrate that a such mechanisms could occur in murine Tcell lymphoma .

T 22 ANALYSIS OF CLONE-SPECIFIC T-CELL RECEPTOR (TCR) GAMMA/DELTA-CHAIN SEQUENCES IN MALIGNANT LYMPHOMAS AND LEUKEMIAS.

M. Kneba, I. Bolz and J. Bertram. Dept. of Hematology and Oncology, University Clinics, Robert Koch Str. 40, D 3400 Goettingen, Germany

The structure of rearranged TCR gamma/delta V - J - junctions was investigated in DNA extracted from 17 patients (6 T-All, 9 peripheral T-cell lymphomas, 2 normal control peripheral blood lymphocytes and the T-cell lines HUT 102 and Jurkat). Rearranged V (gamma/delta) - J (gamma/delta) TCR gene segments were amplified across their clone-specific V-N-(D)-J- junctions by the polymerase chain reaction (PCR) with V- and J- specific primers. Because most of the lymph node biopsy specimen or bone marrow samples, from which the DNA was extracted contained significant amounts of admixed, nonmalignant gamma/delta T-cells, direct DNA- sequencing of the PCR-products yielded unreliable sequence data due to polyclonal V-N-(D)-J- junctions which were coamplified together with the clonal TCR genes. We therefore cloned the PCR- products after ligation in pUC 19 vector DNA and transformation in E. coli DH5a. The sequences of 4-10 cloned and separately analyzed PCR-products from each individual patient or cell line were determined. In the polyclonal controls all analyzed PCR-products differed in their clone-specific V-N-(D)-J- junctions, as expected. In the clonal controls (T-cell lines) and in the T-cell malignancies, several (30-100%) of the cloned isolates contained identical (i.e. clonal) V-N-(D)-J- junctional sequences which are useful as identification sequences for individual T-cell clones. By sequencing a total of 104 TCR-gamma and 67 TCR-delta V-N-(D)-J- junctions, clonality could be demonstrated exclusively in the T-cell lines, in 6/6 of the acute lymphoblastic leukemias and in 7/9 of the T-cell lymphomas. The results were confirmed by temperature-gradient-gel-electrophoresis (TGGE) showing distinct DNA bands only with those PCR-products, which contained clonal (i.e. identical) TCR-gamma/delta V-N-(D)-J-junctions. In summary we demonstrate that coupling of the amplification of TCR-gamma/delta V-N-(D)-J- junctions by PCR, identification of clonal PCR products by TGGE and DNA sequencing is the method of choice for the characterization of clonal TCR-sequences as sensitive and potentially useful diagnostic markers in T-cell malignancies.

T 23 INVOLVEMENT OF THE *BCL-2* AND *c-MYC* GENES IN A UNITED KINGDOM SERIES OF NON-HODGKIN'S LYMPHOMAS.

K.A. Lee[1], M.C.M. Finnegan[1], J.R. Goepel[2], D.A. Winfield[3], B.W. Hancock[1] and M.H. Goyns[1]. [1]Dept. of Clinical Oncology, Institute for Cancer Studies and [2]Dept. Pathology, University Medical School, Sheffield, S10 2RX; and [3]Dept. Haematology, Royal Hallamshire Hospital, Sheffield, S10 2JF, U.K.

The genes that are most often associated with the evolution of non-Hodgkin's lymphomas (NHL) are *BCL-2* and *c-MYC*. These genes were initially identified through studies of low grade follicular NHL and high grade Burkitt lymphoma respectively, but their relevance to other forms of NHL is still unclear. To clarify this question, we have studied these genes in a U.K. series of both low and high grade NHL. We investigated *BCL-2* by using a combination of Southern blot hybridization and polymerase chain reaction (PCR) analysis. The frequencies of rearrangements in our series were 9/20 (45%) in low grade follicular NHL, 1/8 (12.5%) in low grade lymphocytic and 5/19 (26%) in high grade NHL. However, estimation of the high grade value was complicated by the fact that a number of the high grade samples in our series were from patients who had transformed from low grade follicular disease. If the patients were ranked on the basis of whether they had a history of low grade follicular disease then the frequency of *BCL-2* rearrangement remained the same 13/29 (45%), but was only 1/10 (10%) in high grade NHL with no history of follicular disease. It is likely therefore that the presence of rearranged *BCL-2* genes in high grade NHL samples is a marker for previous low grade follicular disease. Southern blot analysis of *c-MYC* revealed no evidence of rearrangement of this gene in any of our NHL even when BamHI-digested DNA samples, which allowed analysis of a 26kb region surrounding the gene, were studied. This included the only sample that we had observed to contain a t(8;14), which indicated that the breakpoint on chromosome 8 in this sample must have been distant to the *c-MYC* gene. By use of a PCR strategy, we were able to identify mutations in exon I of *c-MYC* in 3/19 high grade NHL samples but in 0/21 low grade samples. These data indicated that the frequency of *c-MYC* rearrangements in the U.K. NHL samples was very low and that mutation of the regulatory exon I might be a more common event. We also determined whether any abnormal expression of *c-MYC* might occur. As we had demonstrated that housekeeping gene expression was not a reliable control in Northern blots of NHL RNA [Finnegan et al., 1993, *Leukemia Lymphoma*, In press], we used18S rRNA as a control against which *c-MYC* expression could be measured. Preliminary data from these studies have indicated that more NHL samples may exhibit abnormal levels of *c-MYC* mRNA that would have been anticipated from the above DNA studies.
This work was supported by the Yorkshire Cancer Research Campaign.

T 24 THE EXPRESSION OF Bcl-2 AND c-myc PROTEINS IN LOW GRADE NHL.

U. Martinsson, B. Glimelius, C. Sundström, Depts of Oncology and Pathology, University Hospital, S-751 85 UPPSALA, Sweden.

The expression of the Bcl-2 and the c-myc proteins were investigated in 27 pts with low grade NHL (B-CLL 5 pts, immunocytoma (IC) 5, centrocytic (CC) 6, follicular centroblastic-centrocytic (fCBCC) 5, mucosa associated lymphoid tissue (MALT) 3, hairy cell leukemia (HCL) 3; Kiel classification). The immunohistochemical APAAP technique was utilized on frozen sections of lymph nodes (HCL: spleens). The evaluation was done semi-quantitatively in a light microscope and the intensity was estimated as 1-3+.

In the c-myc stainings, all tumor cells of all 26 patients (one case of HCL not evaluable) were positive. The CC group had statistically significantly more cases with low intensity (1+) than the B-CLL group (5/6 vs 1/5, p<.05). IC and fCBCC: 2/5 each, MALT 1/3, HCL 1/2.

Bcl-2 was expressed by 22/26 pts (1 case of IC not evaluable) on 70-100 % of the tumor cells. Negative cases: B-CLL, IC, CC, fCBCC 1 case each. 1+: B-CLL 2/5, IC 1/4, CC 0/6, fCBCC 2/5, MALT and HCL both 0/3.

The staining intensities were not correlated with serum thymidine kinase or serum LDH levels, stage, indolent vs active disease, or time to active disease for initially indolent cases for any of the two antibodies.

T 25 DETECTION OF CLONAL B-CELL POPULATIONS IN NHL USING PCR.

N. Corbally, D.Devaney, L.Grogan, PA.Dervan, DN. Carney. Depts. of Medical Oncology and Pathology, Mater Misericordiae Hospital & Dept. Pathology, University College Dublin, Dublin, Ireland.

Gene rearrangement has emerged as a precise laboratory aid in the diagnosis and classification of malignant lymphoma. In B-cell non-Hodgkin's lymphomas (NHLs), monoclonality can be demonstrated by the presence of unique rearrangements of the immunoglobulin heavy chain gene (IgH) by Southern blotting.

The aims of this study was to determine the value of two specific polymerase chain reaction (PCR) assays in identifying clonal B-cell populations by IgH gene rearrangements in 65 B-cell NHLs at diagnosis, all shown to have rearranged IgH genes by ' Southern blotting. A major advantage of the PCR is the ability to use small amounts of DNA and DNA extracted from formalin-fixed paraffin-embedded tissue (FFPE).
A single round PCR assay (A), utilised a consensus joining (Jh) region primer in conjunction with a consensus variable (Vh) primer to detect clonal B-cell populations. A second PCR assay (B) incorporated the use of semi-nested primers and two separate rounds of PCR amplification.

In B-cell NHL (n=65) using PCR assay (A), a clonal B-cell population was detected in 50% (follicular, 11/17 (65 %) and diffuse, 21/48 (44%)). Using PCR assay (B) IgH gene rearrangement was detected in 66% of NHL (follicular, 14/17 (82 %) and diffuse, 29/48 (60%)) .
In 20 patients with detectable IgH rearrangements, peripheral blood (PB) and bone marrow (BM) obtained at diagnosis, were assayed for the presence of systemic disease.

Using the simple PCR assay (A),the presence of a circulating B-cell clone in PB/BM was demonstrated in 7/19 (36%) NHL patients (follicular (3/4 (75%) and diffuse (3/15 (20%)). A higher detection rate of systemic disease was detected in these patients using the nested PCR assay (B), 9/20 (45%) (follicular (4/5 (80%)) and diffuse (5/15 (33%)).

Using these assays, clonal B-cell populations could be identified in biopsy specimens in the majority of follicular lymphomas and in over half of diffuse lymphomas. Although the nested primer assay improved the detection rate of IgH rearrangements and appeared to be more sensitive in the detection of minimal disease, the simple PCR assay required much fewer manipulations. Unlike the detection of IgH gene rearrangements using Southern blotting, there are still approximately 40% of B-cell NHL in which we cannot detect monoclonal B-cell populations by PCR using a restricted set of consensus primers. Our failure to detect a malignant clone in all B-cell lymphomas is probably due to the heterogeneity of the targeted Vh/Jh sequences and the frequent occurrence of chromosomal translocations at this locus. By also using more specifically targeted genetic changes in NHL, such as chromosomal translocations (eg.t(14;18), t(11;14), t(8;14)) and detecting them by PCR, all subgroups of NHL may have their own molecular markers.
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T 26 ASSOCIATION OF THE *BCL*-1 REARRANGEMENT IN A SUBTYPE OF NHL.

N. Corbally, D.Devaney, L.Grogan, PA.Dervan, DN. Carney. Depts. of Medical Oncology and Pathology, Mater Misericordiae Hospital & Dept. Pathology, University College Dublin, Dublin, Ireland.

The gene product of the *bcl*-1 oncogene, associated with the t(11;14) chromosomal translocation is now recognised to be a novel member of the cyclin family which plays key roles in regulating the cell cycle. This translocation, appears to be associated with the subtype of lymphoma known as diffuse small-cleaved cell or lymphocytic lymphoma. The *bcl*-1 oncogene has also been shown to be amplified without rearrangement in 30% of head and neck squamous cell carcinomas and 15-20% of primary breast cancers.

The aim of this study was to determine the incidence of the t(11;14) chromosomal translocation involving the *bcl*-1 oncogene, in NHL. Southern blotting was performed on 32 B-cell NHL including 14 of diffuse small cleaved cell subtype, using a *bcl*-1 major translocation cluster (MTC) probe. These samples were also probed for the presence of immunoglobulin heavy chain (IgH) gene rearrangements using a joining region (Jh) probe indicating the presence of clonal B-cell populations.

The presence of *bcl*-1 rearrangement, indicating the presence of the t(11;14) translocation was detected in 6/14 (40%) of diffuse small cleaved cell NHL. There was no evidence of the translocation in 9 patients with low grade follicular lymphomas, (which are frequently associated with the t(14;18) translocation involving the *bcl*-2 oncogene), nor in 2 patients with chronic lymphoctic lymphoma (CLL) in which the t(11;14) was originally identified. The t(11;14) translocation was not found in any of the other intermediate /high grade diffuse lymphoma (n=7) examined. IgH gene rearrangements were detected in all of the above lymphomas examined.

These findings confirm the association of *bcl*-1 with diffuse small cleaved cell lymphomas. The presence of the translocation in a biopsy from NHL may confirm the diagnosis of this subtype of lymphoma. It is known that apart from the MTC breakpoint region, other breakpoints occur within the *bcl*-1 locus which would require the use of additional probes for their identification. The clinical impact of the t(11;14) on the biology and outcome of patients with this disease need to be determined. The involvement of the *bcl*-1 oncogene in the pathogenesis of this type of lymphoma has also yet to be established.

Supported by Cancer Research Advancement Board, Irish Cancer Society.

T 27 DETECTION OF IMMUNOGLOBULIN (IG) GENE REARRANGEMENT IN LYMPHOID MALIGNANCIES OF B-CELL LINEAGE BY SEMI-NESTED POLYMERASE CHAIN REACTION.

Liang R, Chan V, Chan TK, Wong T, Chiu E, Todd D. University Department of Medicine, Queen Mary Hospital, Hong Kong.

The DNA fragments of the complementarity determining region 3 (CDR3) of the Ig gene heavy chain (JH) were amplified by the PCR technique. Two pairs of primers were designed. Either one of the two relatively conserved DNA sequence of 22 base pairs apart from each other, located in the V region [V670 starting at position 666: 5'-ACG GCC GTG TAT TAC TG-3']; VH26 starting at position 688: 5'-AAC AGC CTG AGA GCC GAG GA-3'] was used as the 5' primer. A highly conserved DNA sequence at the 3' end of the J-segment was used as the 3' reversed primer [OL-4 starting at position 799:5'-ACC TGA GGA GAC GGT GAC C-3']. DNA specimens obtained from the lymph node, peripheral blood or bone marrow specimens of 20 patients with B-cell lymphoma, 27 ALL of pre-B (or B) lineage and 19 B-CLL, who all had JH rearrangement by Southern analysis, were studied. Positive PCR analysis was indicated by one or two DNA fragments of sizes between 70 to 140 bp on polyacrylamide gel electrophoresis using the two pairs of primers sequentially (semi-nested) and was found respectively in 40%, 74% and 63% of patients with B-cell lymphomas, ALL of B or pre-B lineage and B-CLL. The sensitivity of the PCR technique was determined by serial dilutions of DNA specimens of known quantity of leukemic cells with DNA specimens from normal control. It can detect with confidence the additional band in a mixture containing 0.01% of leukemic cells. The PCR technique is potentially useful in detecting minimal disease in the peripheral blood and bone marrow of lymphoma patients and residual leukaemia cells in the remission marrow of ALL patients. The applications of this technique in detecting early relapse in ALL, staging lymphoma at diagnosis and identifying tumour contaminated autologous marrow before autologous marrow transplant need to be further assessed.

T 28 *BCL*-2 PROTEIN EXPRESSION IN FOLLICULAR LYMPHOMA AND FOLLICULAR HYPERPLASIA: CORRELATION WITH MT2 IMMUNOREACTIVITY AND THE t(14;18) TRANSLOCATION

D. Devaney, N. Corbally, D. Carney, P.Dervan. Depts. Oncology & Pathology, Mater Misericordiae Hospital, and Dept. Pathology, University College Dublin, Dublin, Ireland.

Diagnostic distinction between follicular lymphoma and follicular hyperplasia is occasionally difficult. Follicular lymphoma frequently shows aberrant MT2 staining within the neoplastic follicles, whereas in contrast, hyperplastic/reactive follicles demonstrate negative staining with MT2. The protein product of the *bcl*-2 oncogene (the gene involved in the t(14;18) chromosomal translocation occurring in 80% of follicular lymphoma) shows a similar pattern of immunostaining.

In this study we assessed the value of *bcl*-2 protein and MT2 immunostaining in distinguishing follicular lymphoma from follicular hyperplasia and correlated the results with t(14;18) chromosomal translocation as detected by PCR.

35 formalin-fixed follicular lymphomas and 16 reactive lymph nodes were immunostained with *bcl*-2 protein and MT2. The major breakpoint region (MBR) of the t(14;18) translocation was evaluated using PCR. 30/35 (85%) follicular lymphoma showed positive staining of neoplastic follicles by both *bcl*-2 protein and MT2. B5 fixed tissue, when available, showed stronger immunostaining. All germinal centres of follicular hyperplasias were negative with both antibodies. 19/33 (57%) of follicular lymphomas, in which PCR amplifiable DNA was obtained, demonstrated the t(14;18) chromosomal translocation.

Both *bcl*-2 and MT2 immunostaining show a high degree of sensitivity in distinguishing follicular lymphoma from follicular hyperplasia even in the absence of demonstrable t(14;18) translocation in the MBR. These antibodies may provide useful diagnostic tools in equivocal diagnoses in the routine histopathology laboratory.

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T 29 CHROMOSOME CHARACTERIZATION OF A CASE OF T CELL-RICH B-CELL LYMPHOMA

Roberta La Starza, Brunangelo Falini, Daniela Falzetti, Caterina Fania, Massimo F. Martelli, Cristina Mecucci - Institute of Hematology, Policlinico Monteluce, University of Perugia, 06100 Perugia, Italy

B Cell Lymphoma enriched in T cells has been recently defined as a distinct entity. Differential diagnosis from Hodgkin's disease may be difficult on histological ground especially for the presence of large atypical cells with Reed-Sternberg like features. Immunophenotyping, however, is helpful to show positivity for B cell antigens and lack of expression of typical Reed-Sternberg associated antigens such as CD15 and CD30. As far as we know chromosome findings of this entity are still unknown. We report on cytogenetic results of a typical case of T cell rich B-cell lymphoma. The patient, a 52 years old woman, presented with a stage IV disease; diffuse lymphoadenomegaly, mediastinal enlargement, consistent splenomegaly, and anemia (Hb 8.9 g/dl). Hypergammaglobulinemia with a monoclonal lambda component was also present. Immunohistochemistry showed positivity for CD19, CD20, and CD22. CD30 was negative. Neoplastic B cells were actively proliferating(> 50% Ki67 positive elements). Cytogenetic investigations were performed on lymph node biopsy at diagnosis. Metaphases after 48 hour cultures were G-banded with Wright stain. Eighty per cent of karyotypes showed near-tetraploidy(modal number = 83). A 14q+ marker was present in all abnormal metaphases. Two out of ten abnormal metaphases also showed a 17p+ marker due to the presence of extramaterial on the short arm of chromosome 17. Cytogenetic results in this case provide new insights in the biology and differential diagnosis of T cell-rich B-cell lymphoma. First, the high degree of ploidy, namely near-tetraploidy, may be attributed to the large atypical cells by analogy with the tetraploidy typical of Reed-Sternberg cells in Hodgkin's disease. Moreover a 14q+ marker is unequivocally associated with B cell lymphoma, while it is unexpected in Hodgkin's disease.

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T 30 B-CELL NON HODGKIN'S LYMPHOMA WITH MULTIPLE BREAKPOINTS SPECIFIC FOR B-CELL MALIGNANCIES.

I. Wlodarska[1], M. Stul[1], C. Mecucci[1], Ch. De Wolf-Peeters[2], J.J. Cassiman[1], H. Van Den Berghe[1]. [1]Center for Human Genetics and [2]Department of Pathology, University of Leuven, Leuven, Belgium.

The generally accepted hypothesis of multistep carcinogenesis assumes that the sequence of genetic events occurs during cancer development. We report here a case of high grade B-cell Non Hodgkin's lymphoma which is in keeping with this hypothesis. Cytogenetic analysis performed at the time of high grade lymphoma diagnosis showed the presence of three related cell clones in the same malignant cell population:

I - 48XY,dup(1)(q32q21),t(3;18)(q29;q21),der(3)t(3;18),+18
II - idem, t(X;21)(p11;p11),der(14)t(8;14)(q13;q32)
III - idem, t(11;14)(q13;q32)

The presence of three cell clones showing different degrees of karyotype complexity but all carrying the same chromosome anomalies /dup(1q),t(3;18),+18/ may reflect the evolution of malignant karyotype during the progression of disease. The finding of three chromosome translocations with breakpoints typical for B-cell malignancies e.g. t(3;18)(q29;q21), t(8;14)(q13;q32) and t(11;14)(q13;q32) suggests the involvement of more than one oncogene in development of the present lymphoma. Molecular investigation of translocations in this case are being performed by using a FISH approach as well as by testing BCL1, BCL2 and FVT1 probes.

T 31 IN SITU EXPRESSION OF THE IL-1-alpha AND TNF-alpha GENES BY REED-STERNBERG CELLS (RSC) IN HODGKIN'S DISEASE (HD).

L. Xerri, F. Birg, R. Bouabdallah, V. Guigou and J.Hassoun. INSTITUT PAOLI-CALMETTES , MARSEILLE, FRANCE.

The histopathologic pattern of tissues involved by Hodgkin's disease (HD) suggests excessive activation of environmental cells by cytokines released by Reed-Sternberg cells (RSC), which are considered as the neoplastic component of HD lesions. This hypothesis has been supported by many studies performed in vitro using HD cell lines. In this study, we have tried to demonstrate the cytokine-producing cells in an environment as close as possible to the in vivo conditions, using in situ hybridization onto frozen sections of HD samples. [35-*S]-labelled single-stranded RNA probes were prepared by transcribing human cDNA fragments of the TNF-alpha and IL-I alpha genes subcloned into appropriate vectors. A total of 19 specimens of HD lesions, including 7 cases of nodular sclerosing (NS) type and 12 cases of mixed cellularity (MC), were tested with both types of probes. Clinical stages included stage I (6 cases), stage II (4 cases), stage III (6 cases) and stage IV (3 cases). TNF-alpha and/or IL-1 alpha expression was observed in 12 among 19 HD cases. However, neither the histological type nor the clinical status of the patients was correlated with the profile of cytokine secretion. Most of the cytokine-producing cells could be identified as RSC due to their morphological appearance. In 3 cases, simultaneous analysis by immunohistochemistry and in situ hybridization showed that IL-I alpha/TNF-alpha mRNA-producing cells simultaneously expressed the CD30 antigen, thereby confirming the Reed Sternberg nature of these cells.

T 32 HETEROGENEITY OF REARRANGED T-CELL RECEPTOR V-ALPHA AND V-BETA TRANSCRIPTS IN TUMOR INFILTRATING LYMPHOCYTES FROM HODGKIN'S DISEASE AND NON HODGKIN'S LYMPHOMAS.

L. Xerri, M.-P. Mathoulin, F. Birg, R. Bouabdallah, A.-M. Stoppa and J. Hassoun. INSTITUT PAOLI-CALMETTES, MARSEILLE, FRANCE.

Hodgkin's disease (HD) is histologically characterized by the presence of Reed-Sternberg cells (RSC) associated with reactive cells, which include numerous T-lymphocytes. Some forms of B-cell non Hodgkin's lymphomas (B-NHL) also contain a rich T-cell population. Previous studies have suggested that the T-cell receptor (TCR) repertoire of tumor infiltrating T-lymphocytes (TITL) acting specifically against tumor-related antigens should be restricted. We have explored the possible existence of such an immunologic response of TITL against RSC or neoplastic B-cells by investigating with the polymerase chain reaction, the expression of variable (V) region genes of TCR alpha and beta chains by intratumoral lymphocytes infiltrating biopsy specimens. 7 HD and 3 B-NHL biopsy samples were included in the study. HD cases were classified as nodular sclerosing (n=4), mixed cellularity (n=2), and lymphocyte predominance (n=1). B-NHLs were high grade, diffuse large cell lymphomas, selected on the basis of a rich T-cell reactive content. Peripheral blood lymphocytes from 3 patients with HD and from 2 healthy subjects were simultaneously analyzed. Primers specific for 18 different V-alpha and 21 V-beta families were used. In all of the HD and NHL cases, TITL showed an unrestricted pattern of expression similar to the repertoire observed in peripheral blood lymphocytes. PCR products of the 18 V-alpha and 21 V-beta families from 1 HD case were then radioactively labelled and separated on polyacrilamyde gels. Autoradiography showed that amplified products from every family were actually heterogeneous in size with subtle differences suggesting the presence of several T-cell clones simultaneously using each V-family. In none of the V-families was observed any predominant band that could correspond to specifically activated T-cell clone. Despite the limitations of such experiments, the apparent lack of selection of a single or of a limited number of T-cell specificities in the affected tissues does not support the existence of an *in vivo* immunologic interaction between TITL and antigens related to RSC or neoplastic B-cells.

T 33 STAGE IV EBV NEGATIVE BURKITT LYMPHOMA (BL) IN A CHILD WITH CONSTITUTIONAL t(1;6) (p36;q22) TRANSLOCATION.

C.Berger, D.Frappaz, H.Sobol, C.Charrin, I.Philip, V.Combaret, H Plauchu, M.Brunat-Mentigny, T. Philip.
Pediatric Department. Centre Léon Bérard, 28, rue Laënnec, 69373 LYON. France

Approximately 1 of 150 newborn has a constitutional chromosome anomaly (CCA), of which one third are balanced structural rearrangements. The incidence of BL in children is $6/10^6$children/year in France. The risk that a BL occurs in a child with a CCA is thus limited. No case has been reported so far.

Case report : Marc C. is a 4 years old child with a stage IV bone marrow positive abdominal BL. The fine needle aspiration of the abdominal mass shows typical BL cells, CD19+ CD20+. Malignant cells fail to grow in the liquid culture system. Karyotype shows a t (1;6) (p36;q22) in initial bone marrow sample and in peripheral lymphocytes. Molecular biology study is currently underway on tumoral cells. There is no familial history of either cancer or malformation. Karyotype reveals the same translocation in his father and unique brother. The child is currently in CR 2 months off therapy (LMB 89 group C).

Discussion: No case of t(1;6)(p36;q22) CCA associated with a malignancy have been reported so far. However, CCA involving chromosome 1p36 is reported in a 9 month old infant with neuroblastoma. The breakpoint 1p36 was never described in BL tumors but aberration in the 1p32-36 region is reported in nearly 13% of lymphoma, associated in 16% with aberrations of 6q21-25. The breakpoint 1p36 was also reported in neuroblastoma and uterine leïomyosarcoma. The c-fgr oncogene (which may have a highly specialized function in hematopoietic cells) is located next to this breakpoint. A c-fgr mRNA increase has been found in EBV-negative BL cell lines .

The 6q22 is also an interesting breakpoint, since a duplication (dup(6) (q22 q26)) was described in an ALL3, and a deletion (del (6) (q15 q22)) in a BL. Moreover, at least 8 observations involving to 6q22 region were reported in NHL. The c-myb oncogene whose expression is associated with cell proliferation is mapped to chromosome 6q 22-24.

Conclusion : The occurence of a BL in a boy with CCA t(1;6) (p36;q22) may not be random, and may involve oncogenes that are responsible for tumorigenesis.

T 34 KARYOTYPE AND PROGNOSIS IN NON-HODGKIN LYMPHOMAS

Ch. Marosi, H. Pirc-Danoewinata, A. Chott, E. Onderka, E. Schlögl, U. Jäger, F. Thalhammer, GG. Steger, G. Locker, I. Michl, H. Hanak, R. Heinz. Division of Oncology, Departement of Internal Medicine I, 1090 Vienna, Austria

Successful cytogenetic analysis was available from 70 adult patients with various biopsy proven Non-Hodgkin lymphomas (NHL) prior to chemo- or radiotherapy. A t(14;18)(q32;q21) was found in 24 patients, including 5/9 (30%) with centroblastic (CB), 6/8 (75%) with centrocytic (CC) and 3/7 (43%) with CBCC lymphoma. According to previous studies, increasing karyotypic complexity correlated with high grade histology, aggressive clinical course and shortened survival. Regardless to histologic diagnosis, in NHL where aberrations of chromosome 7 (n=14) or deletions of the short arm of chromosome 17 (n=14), the affected patients showed a high tumor burden, high serum LDH (median 657+167 U/l). However a significant difference of survival times could not be ascertained. Additional aberrations of chromosome 3, mostly del(3)(p21-25) were found in 14/19 (74%) of these patients. Three patients with aberrations of 11p with possible involvement of the gene coding for CD44 suffered from abdominal bulky tumors , showed poor response to therapy and short disease free survival.

T 35 PHORBOL-12,13-DIBUTYRATE IMPROVES QUALITY OF CYTOGENETIC PREPARATION IN LYMPHOID MALIGNANCIES.

H. Pirc-Danoewinata, E. Onderka, R. Heinz, F. Thalhammer, G. Porenta, Ch. Marosi. Division of Oncology, Departement of Internal Medicine I, 1090 Vienna, Austria

Biopsies of enlarged lymph node in the clinical situation of suspicion of malignant lymphoma is usually done before histologic confirmation when neither cell lineage nor histologic subtype are available. In this constillation a mitogen should be able to activate B- and T-cell proliferation without any toxity for lymphatic cells or overstimulation of fibroblasts.
Phorbol-12,13-dibutyrate (PDBu) was taken as a tumorpromotor to activate a variety of cellular responses, and therefore also cell proliferation. The effect of PDBu alone and PDBu together with A23187 (A), a Ca2+-Ionophore inducing Ca^{2+} mobilization, was investigated on lymph node biopsies from 13 consecutive patients under suspicion of malignant lymphoma and acute lymphatic leucemia. We compared quality and quantity of mitoses obtained in unstimulated and stimulated short-term cultures under standard conditions with final concentrations of 5nM for PDBu alone and 10nM PDBu in combination with 500nM A. There was no evidence of lymphotoxity of neither P or PA. Focussing of high quality of analyzable metaphases best results were regularly found in 24h culture followed by 48h cultures stimulated with PDBu alone ($p < 0,05$) and 24h culture without any agents within a patient. Stimulation with PA was inferior to P and of no further benefit.

T 36 UNCOORDINATED TYROSINE PROTEIN KINASE (TPK)/PROTEIN PHOSPHATASE(PP) IN MALIGNANT LYMPHOMA CELLS.

A.A. Hakim. Molecular Biology and Biochemistry.
180 Longwood Drive. Kankakee. Illinois 60901. U.S.A.

Malignant cell proliferation, progression, invasion and metastasis are modulated by a cascade of hydrolases(Hakim Experientia 41:1579-1584, 1985) which cause the release of serine, tyrosine and threonine residues essential in celluler glycosylation (Hakim Proc.Exp.Biol.Med.185: 158-176,1987)/ Phosphrylation mechanisms needed to modulate growth factor receptors(GFR) and oncogene cellular functions (Hakim J.Surg. Oncology 40: 21-31, 1989, Diagnostics 27:30-37,1989). Guanine nucleotide-binding proteins (G-Protein) a molecular switch system that modulates GTP-binding activation/deactivation indyced upon lysis of GTP by the intrinsic GTPase activity a cycle that sort and amlify transmembrane signals transmitted through PTK oncogenes/growth factor receptor (GF/GFR) (Hakim GTP-Binding Protein(G-Protein) Couples RAS P-21 oncogene and Growth Factor Receptors (EGF-R,PDGF-R)in Signaling Carcinoma Cell Aggressiveness. 8th. International Conference on Second Messengers & Phosphoproteins D113 T, 4 August 1982). The aim of the present study was to investigate the relationship between PTK/PP cycle and P53 a 53-Kd nuclear phosphoprotein function. In-vitro cultured K-562 cells derived from human malignant lymphoma a Natural Killer (NK)-specific targets. P53 was monitored by staining with MAb1801 which recognizes a denaturation-resistant epitope in human P53 located between amino acids 32 and 79, revealed by biotinylated anti-mouse Ig and peroxidase-conjugated streptavidin/0.01 mmol/L 3-3'-diaminobenzidine-2 mmol/L hydrogen peroxide in 0.05% Tris-HCl buffered at pH 7.6. P53 is highly conserved in vertebrates and regulates entry into and progression through the normal cell cycle, being induced during the transition from G0 to G1 in the cell cycle. In normal fetal and adult tissues P53 is expressed in low level and has a very short half-life. In cultured cells increased levels are associated with a mutated form of the protein or with stabilization of the protein in a complex with viral antigen, immortalize cells in vitro, and in conjunction with an activated Ras oncogene, P53 produces a fully transformed phenotype in primary cultured fibroblasts. The Wild-type protein has a tumor suppressor action. Thus increased levels of P53 indicate increased stability and higher-steady-state levels of the protein serving as a marker for the mutated form of P53 protein in human malignant lymphomas. K-562 resistance to NK-killing action correlated directly with mutated P53-PTK-activities, and indirectly with cellular wild P53 and protein phosphatase levels. Phosphorylation of PTK maintains the enzyme in an active form (ATP.Protein) Complex while protein phosphatase and wild P53 produce inactive phosphorylated state. The latter state has been demonstrated in lung cancers, breast cancers, Burkitt lymphomas and chronic lymphocytic leukemias.

T 37 CELL KINETIC STUDIES OF NON-HODGKIN´S LYMPHOMAS BY *IN VIVO* IDURD INCORPORATION AND FLOW CYTOMETRY.

M. Erlanson, J. Lindh, G. Landberg, G. Roos, Departments of Oncology and Pathology, University of Umeå, Umeå, Sweden.

By flow cytometric analysis of *in vivo* incorporated thymidine analogs detailed tumor cell kinetic evaluation can be performed with important clinical implications. In the present study 32 non-Hodgkin´s lymphomas were investigated.

The patients got an infusion of 100mg iododeoxyuridine (IdUrd) approx. 4 hours prior to tumor biopsy. A cell suspension was fixed and stained with an FITC labelled anti-IdUrd antibody after denaturation of DNA. After addition of propidium iodide flow cytometric analysis was performed and S-phase fraction (SPF), labelling index (LI), S phase duration time (Ts) and potential doubling time (Tpot) were determined.

Fortyfive patients initially got IdUrd of which 32 were evaluable. The deficit was due to sparse material or aneuploidy. The results are listed below.

		Low grade (n=15)	High grade (n=17)	p value
SPF (%)	range	0.7 - 9.8	2.7 - 25	
	median	1.7	6.4	<0.001
LI (%)	range	0.8 - 9.7	2.6 - 31.4	
	median	3.7	7.4	0.003
Ts (h)	range	4.3 - 10.5	4.2 - 20.1	
	median	7.8	9.9	0.01
Tpot (d)	range	1.5 - 32.9	0.8 - 18.2	
	median	7.3	3.7	0.044

The results show that it is possible to study the dynamic cell proliferation in lymphomas with single biopy technique using *in vivo* IdUrd incorporation. As expected, low and high grade lymphomas differed concerning SPF, LI and Tpot and interestingly high grade cases showed somewhat longer Ts times. The clinical significance of these parameters remains to be determined.

T 38 Immunophenotyping of Aggressive Non-Hodgkin's Lymphoma and response to treatment.

N. Mokhtar, H.M. Khaled, S. El-Husseiny, H. Taha, N. El-Bolkainy, N. Gad El-Mawla, I. Magrath*, and E.S. Jaffe*.

NCI, Cairo, Egypt.
* NCI, Bethesda, USA.

A total of 52 cases of grades II and IIIaggressive NHL who were treated with the BECOP regimen at Cairo NCI were included in the study. Cases were diagnosed histologically according to the Working Formulation.

A panel of monoclonal antibodies was applied on paraffin embedded formalin fixed sections or cytospins for common leucocyte antigen (LCA), T-cell markers (UCHL, and Leu 22), B-cell markers [L-26, Kappa, and Lambda], Monocyte marker [Leu M_1], and activated cell marker [Ki-1].

No significant difference was detected between grade of the tumor and immunophenotyping as both grades II and III were of the B phenotype with Kappa predominance in 86.1% and 81.2% respectively. Mixed cell, large cell, and immunoblastic cases showed high residual T-cell population in both B and T tumors. CD_{30}, a marker of cell activation was strongly positive in one case of Burkitt's, 3 cases of immunoblastic lymphoma all of T phenotype, and one case of large cell lymphoma. Selected cases of mixed and immunoblastic lymphomas were all negative for Leu M_1 marker.

A total of 41/52 patients (78.8%) had complete remission (CR) under BECOP regimen, 88% of them were of the B cell and 12% of the T cell phenotype. Five patients had no CR four of them were of the B cell phenotype. Cases with high residual T lymphocytes all had CR on treatment, while those with positive Ki-1 had either stable disease or progression on therapy. Three patients died of drug related toxicities while on treatment, all had B-cell phenotype, while the remaining 3 were lost to follow up.

It could be concluded that there is no relation between immunophenotype and response to therapy. However, the rich T cell infiltrate may denote a host reaction to patients' benefit.

T 39 FLOW CYTOMETRIC DNA ANALYSIS OF 125 NON HODGKIN'S LYMPHOMAS; A COMPARISON WITH MICRODENSITOMETRIC STUDY.

J.Vučković, M.Marušić, G.Forempoher et al. Department of Haematology and Oncology, DZ "Dr.Petar Vitezica" 58000 Split, Croatia., KBC "Firule" 58000 Split, Croatia

DNA Flow Cytometry was done on paraffine embedded lymh nodes taken before therapy in 125 Non-Hodgkin lymphoma (NHL) patients. Frequency of aneuploidy was 27 % (34/125). There was no significant difference between frequency of aneuploidy in low-grade NHL (LG-NHL), 31 % (15/48) and in intermediate+high-grade NHL (HG-NHL) 25 %, (19/77). In LG-NHL aneuploidy appeared to have adverse effect on survival (p=0,056-0,08). In Hg-NHL as well as in the whole group ploidy status did not influence the survival. Increased proliferative activity, expressed as S+G2M > 6 % had adverse effect on survival in the whole group. The median survival for high-proliferative group (HP) was 27 months and for low-proliferative (LP) it was 72 months, p=0,02. Inside LG-NHL, the difference in survival between HP and LP groups was highly significant (p< 0,01), while in HG-NHL such difference between HP and LP lymphomas was not found. Simmilar resultes ragarding proliferative activity and survival were obtained by microdensitometric study. Values for S+G2M measured by two methods showed high degree of correlation, r=0,89 p < 0,01.

T 40 ANALYSIS OF THE PROTEIN SYNTHESIS PROFILES OF CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS, NORMAL B-LYMPHOCYTES AND LEUKAEMIC PHASE CELLS OF LOW GRADE LYMPHOCYTIC NON-HODGKIN'S LYMPHOMA.

F.K. Saunders[1], R.M. Sharrard[1], J.R. Goepel[2], D.A. Winfield[3], B.W. Hancock[1] and M.H. Goyns[1]. [1]Dept. of Clinical Oncology, Institute for Cancer Studies, and [2]Dept. of Pathology, University Medical School, Sheffield, S10 2RX; and [3]Dept. Haematology, Royal Hallamshire Hospital, Sheffield, S10 2JF, U.K.

The molecular lesions responsible for the evolution of chronic lymphocytic leukaemia (CLL) remain obscure, as does the identity of its non-malignant counterpart. Futhermore, although CLL is often included in classification schemes of non-Hodgkin's lymphomas (NHL) as a form of low grade disease, it is still unclear whether CLL can really be regarded as the same as the leukaemic phase of low grade lymphocytic NHL. To address these questions we have analysed the protein synthesis profiles of both malignant and normal lymphoid cells by 2D-gel electrophoresis. Initially protein synthesis was analysed in leukaemic cells from 10 CLL patients by 2D-gel electrophoresis of ^{14}C-labelled proteins. There appeared to be only minor differences between each of the CLL samples, but there was evidence that the level of expression of a few of the proteins might have correlated to the stage of disease. In particular one protein (approx size 85kD) was observed to show significantly enhanced synthesis in the more aggressive forms of the disease, and we are currently attempting to characterize this protein. Comparison of the CLL samples with populations of normal B-lymphocytes demonstrated marked differences in protein synthesis between the leukaemic cells and the non-malignant cells. We subsequently used the fluorescence activated cell sorter to separate CD5+ve from CD5-ve B-lymphocytes, but observed that the protein synthesis profiles exhibited by these two populations were essentially the same, and both were very different to that observed in CLL cells. We have also analysed B-lymphocytes from tonsil and amniotic cord blood, and it appeared that the pattern of protein synthesis most similar to that observed in CLL cells occurred in CD5+ve B-lymphocytes from cord blood. Thus CLL may represent a malignant transformation of foetal B-cells. We have also recently begun to analyse leukaemic phase cells from patients who originally presented with low grade lymphocytic NHL, and preliminary data has suggested few differences between these cells and the CLL samples.

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T 41 IMMUNOPHENOTYPING OF LOW GRADE B-CELL LYMPHOMA IN BLOOD AND BONE MARROW

M. Schwonzen, C. Pohl, T. Steinmetz, W. Seckler, B. Vetten, J. Thiele*, D. Wickramanayake, and V. Diehl

Klinik I für Innere Medizin, D-5000 Köln 41
*Institut für Pathologie, D-5000 Köln 41

Based on a hypothetical model of B-cell ontogeny, non-Hodgkin's lymphomas (NHL) of B-cell lineage are considered as a malignant proliferation of particular differentiation stages in lymphopoiesis. According to this theory antigen expression patterns for defined B-cell lymphoma subtypes were examined by immunophenotypic examinations of peripheral blood and/or bone marrow (BM), involved by low grade B-cell non-Hodgkin's lymphomas.
The following markers were used for immunocytology (APAAP-technique) of blood and/or BM smears: CD19, CD5, CD10, CD11c, CD14, CD21, CD22, CD23, CD25, CD38 and TdT.
Results were compared with the results of cytomorphological and histopathological examinations in 133 adult patients. Sixtynine cases of chronic B-lymphocytic leukaemias (B-CLL), 16 centrocytic (CC), 14 centroblastic/-centrocytic (CB/CC) lymphomas, 15 immunocytomas (IC), 10 cases of hairy cell leukaemia (HCL), four prolymphocytic leukaemias (PLL), two B-CLL in transformation, one splenic lymphoma with villous lymphocytes (SLVL), one hairy cell leukaemia variant (HCL-V), and one lymphocytic lymphoma (LC) were classified according to the Kiel- and or FAB-classification. Leukaemic disease was found in 105 cases. All cases tested showed CD19, but no TdT expression. Every case of HCL had a distinct phenotype with expression of CD11c, CD22, and CD25 and the lack of CD5 and CD23 antigens. In all other NHL cases a very heterogenous expression of CD-antigens with no significant correlations to the cytomorphological subtypes was found. The expression of CD5 is a frequent but inconstant finding in lymphoproliferative diseases other than B-CLL, so 50% of CB/CC, 75% of CC and 80% of IC were CD5 positive. Our results indicate, that, with the exception of HCL, immunophenotyping does not support the cytomorphological classification of low grade NHL of B-cell lineage in blood and/or BM.

T 42 SERUM SOLUBLE INTERLEUKIN-2 RECEPTOR LEVELS MAY BE ASSOCIATED WITH CLINICAL DISEASE STATUS AND HISTOPATHOLOGICAL GRADE IN NON-HODGKIN'S LYMPHOMA.

R. Or, A. Polliack, I. Kalickman, V. Barak. Immunology Laboratory, Department of Oncology, Lymphoma-Leukemia Unit, Department of Hematology, Hadassah University Hospital, Jerusalem, Israel.

Serum soluble Interleukin-2 receptor levels (sIL-2R) were measured in 121 patients (pts) with non-Hodgkin's lymphoma (NHL) and in 30 pts with chronic lymphocytic leukemia (CLL). Sera collected from 32 normal volunteers and 18 patients with infection or a varitey of non malignant hematological disorders served as controls. A small number of patients with Hairy Cell Leukemia (HCL) and Hodgkin's disease (HD) were also studied. NHL patients were classified according to their clinical status as "active" (82 pts) or "non-active" (39 pts) and CLL according to the stage of their disease. NHL patients were also further classified as low (55 pts), intermediate (38 pts) and high grade malignancy (28 pts), according to the working formulation scheme. A significant difference was found between the high levels of sIL-2R in patients with "active" disease and the lower levels in patients with quiescent or responsive disease. Significantly different high levels were found in patients with aggressive (intermediate and high grade) lymphomas as opposed to low grade lymphoma and CLL. In CLL itself higher levels of sIL-2R were seen in more advanced disease than in early disease. Thirteen patients with active Hodgkin's disease (HD) had moderately elevated sIL-2R levels, similar to those recorded for patients with infections and some non-malignant hematological disorders while another 13 HD patients in remission, had normal levels comparable to those recorded in normal controls. Extremely high levels of sIL-2R were seen in 2 patients with HD with severe viral infections and levels approaching those seen in HCL, were noted (20-30,000 u/ml). This study demonstrates that sIL-2R levels can serve as a reliable indicator of disease activity in patients with NHL and may predict progression of disease or response to therapy, particularly in agressive NHL, provided that the test is not performed at the time of active infection or inflammation.

T 43 DETECTION OF MEMBRANE-BOUND AND SOLUBLE IL 2 RECEPTORS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES.

G. Mantovani, A. Macciò, S. Esu, L. Contini, P. Lai, E. Turnu, A. Balestrieri and G.S. Del Giacco. Department of Medical Oncology, Clinical Immunology and Internal Medicine, University of Cagliari, 09124 Cagliari, Italy

The aim of the study was to evaluate the expression of membrane-bound IL 2 R, the p55 chain (Tac), as assessed by anti-Tac monoclonal antibody (mAb), on unstimulated and PHA-stimulated (at 3 days and at 7 days) peripheral blood mononuclear cells (PBMC), to correlate it with the levels of soluble IL 2 R in serum and in culture supernatants from unstimulated and PHA-stimulated PBMC, and furthermore the proliferative response to PHA, to IL 2 and to PHA plus IL 2 of the PBMC from patients (pts) with haematological malignancies. Furthermore, the proliferative response to PHA, to IL 2 and to PHA plus IL 2 of PBMC of pts with haematological malignancies was evaluated in the absence and in the presence of anti-Tac mAb, to verify the inhibition induced on the PBMC proliferation by anti-Tac mAb. Fifteen pts have been studied: 4 Hodgkin's Lymphoma (HL), 2 with active disease and 2 in clinical remission, 4 non-Hodgkin's Lymphoma (N-HL), 2 with active disease and 2 in clinical remission, 5 Hairy Cell Leukemia (HCL), 1 Chronic Myelogenous Leukemia (CML) and 1 Chronic Lymphocytic Leukemia (CLL). Nine healthy age-sex-matched subjects served as controls. We have found a significant decrease of mitogen-and IL 2-stimulated proliferation of PBMC of pts with haematological malignancies as compared to healthy controls, high values of membrane-bound Tac on unstimulated PBL of HL, N-HL, CML and particularly of HCL, whereas low values of membrane-bound Tac on PHA-stimulated cells in active HL and N-HL were found. Increased serum levels of soluble IL 2 R in active HL, N-HL and in HCL were shown. Therefore, taking into account our previously reported data on defective IL 2 prodution mainly in HL, our present results can support the rationale for more extensive clinical trials with IL 2 in haematological malignancies.

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T 44 INTERLEUKIN-5 IN HUMAN B-CELL TUMORS.

M. Samoszuk, V. Nguyen and E. Ramzi. Pathology Department, University of California, Irvine, California, USA

Interleukin-5 is a T-cell derived cytokine that promotes growth of eosinophils and B-cells in the mouse. To date, there has been little evidence that interleukin-5 also plays a role in the growth or differentiation of human B-cells. Using a polyclonal rabbit antibody directed against human interleukin-5, we developed an immunohistochemical procedure for specifically detecting human interleukin-5 protein in cytopreparations of cryopreserved cells. The procedure was first validated on various positive and negative control specimens (8 cases of Hodgkin's disease with eosinophilia; the ARH-77 and Sp 2/0 cell lines; and 8 cases of benign lymphoid hyperplasia). Following validation of the assay, we tested 12 B-cell tumors of varying types for the presence of interleukin-5. The cells from 3 cases of small lymphocytic lymphoma and 2 cases of large cell lymphoma had modest staining for interleukin-5. There was intense cytoplasmic and membranous staining for interleukin-5 in the cells from 3 cases of small cleaved cell lymphoma and 4 cases of pre-B cell acute lymphocytic leukemia. When analyzed by an mRNA polymerase chain reaction, none of the B-cell tumors also contained detectable mRNA coding for interleukin-5. We conclude that certain human B-cell tumors contain varying amounts of exogenously synthesized interleukin-5 that is detectable by immunohistochemistry. This finding suggests that interleukin-5 could play a role in regulating the growth of some neoplastic human B-cells.

T 45 PROSPECTIVE STUDY OF SOLUBLE IL2 RECEPTOR, IL6 AND IL10 IN NON HODGKIN LYMPHOMA.

V. Ribrag, M. Pallardy, J. Bosq, P. Brault, R. Roger, I. Joab, C. Bohuon, M. Hayat. Institut Gustave Roussy, rue Camille Desmoulins, 94805 Villejuif Cedex - France.

Serum levels of soluble IL2 receptor (R-IL2), IL6, IL10 were prospectively mesured in 14 normal subjects and 28 patients (pts) with Non Hodgkin's Lymphomas (NHL). Immunohistochemical study with anti CD25 and anti IL10 Moab were also performed in biopsy samples of these 28 NHL pts. All these NHL were of intermediate or high grade (WF). Sixteen were B-NHL and twelve were T-NHL. Serum IL6, IL10 and R-IL2 levels were assessed at diagnosis and before each course of chemotherapy. We used an ELISA technic for the quantification of R-IL2, IL6 and IL10 levels. R-IL2 was detectable in all serum samples tested and was different from normal subjects as compared to NHL (156 pM vs 37 pM, $p < 0.01$). Furthermore T-NHL had higher R-IL2 levels at diagnosis compared to B-NHL (223 pM vs 105 pM, $p < 0.01$). During treatment R-IL2 level decreased in responsive T-NHL pts (223 pM vs 133 pM after chemotherapy, $p < 0.05$). We did not observed any significant changes in R-IL2 level during chemotherapy in responsive B-NHL (105 pM vs 117 pM). Immunohistochemical study showed a well correlation between the anti CD25 staining and R-IL2 levels in serum. Malignant T-cells were highly CD25 positive in pts with a high R-IL2 level. On the contrary, IL6 level was low in all samples tested (Normal subjects, T and B- NHL) and was not affected by response to therapy in NHL's. Serum IL10 and immunohistochemical stainings with anti IL10 Moab are currently under investigation. Our results showed that R-IL2 is elevated in T-NHL and is correlated with tumor burden and response to therapy. Despite the fact that R-IL2 was elevated in B-NHL, we have not observed any modification of seric R-IL2 level during chemotherapy whatever a complete remission was obtained. Our results suggest that R-IL2 could be a usefull tumoral markor in T-NHL but not in B-NHL.

T 46 INTERLEUKIN-2 THERAPY IN PATIENTS WITH MALIGNANT LYMPHOMA.

A.A.Umnyashkin, F.M.Abdullayev, R.F.Umnyashkina, S.M.Abdullayeva. Immunology and Genetic Department, Institute of PD, Quarter 25I4, Zardabi Street, Baku 370II8, Azerbaijan Republic

Interleukin-2 (IL-2), produced by stimulated lymphocytes, is thought to hold promise in the treatment of malignant lymphoma. 28 patients (mean age I9,5 years) affected by malignant lymphoma were studied. All recieved intravenous IL-2 (30,000 to 70,000 U/kg) every 8 hours for 9 to I2 days. All aptients had major adverse reactions to IL-2, but only I had to withdraw from the trial. In addition to multiple laboratory abnormalities, at least 80% had fever, chills, malaise, cough, anorexia, hypotension, or skin rash. Overall clinical improvement was modest. The I5 patients improved dramatically during therapy but relapsed within 8 weeks. 7 patients had a 4-month remission, the increase of a number of CDI, CD2I lymphocytes, PHA-induced blastogenesis and normalization of CD4/CD8 indices was detected. Theseoutcomes, especially the side effects, are disappointing for a therapy that once held high hope. On the basis of these results, if it's possible to conclude that perhaps longterm therapy with lower doses of IL-2 in patients with malignant lymphoma will prove more valuable.

T 47 NK AND LAK ACTIVITY IN NON-HODGKIN LYMPHOMAS: EFFECT OF THE CHEMOTHERAPY.

F. Franzin, S.Basile*, G. Presani*, A. Luchesi*, S. Perticarari*, P. Tulissi, M. Moretti and G. Pozzato. Inst. of Patologia Medica, University School of Medicine and *Laboratory of Children Hospital, Trieste, Italy.

Non-Hodkgin Lymphomas (NHL) are neoplastic disorders of the immune system with an initial good response to therapy but an high rate of relapse. Since immunodeficiency seems to be a determining factor for the development of NHL, the present study was planned for defining whether a similar condition occurs after chemotherapy making easier the relapse. Twenty subjects affected by different NHL (Groups C, D, E and F of WF) were recruited in this study. Patients with leukemic syndrome were previously discarted. Immunophenotype was B in 18 and T in 2 cases. The patients were studied before and after 2 types of chemotherapeutic regimens (CEOP or MACOP-B). Nine normal subjects were used as controls. At the end of the therapy all the patients but four obtained complete remission. Peripheral blood mononuclear cells (PBMC) were purified by Ficoll sedimentation and monocytes were discarted by plastic adherence. Cytotoxic studies were performed with a flow cytometric method by determining the light scattering of target cells (previosly incubated with Propidium Iodide) killed by lymphocytes. NK activity was obtained by using K562 cell line as target, and LAK activity with NALM6 cell line (NK resistant). Results were expressed as percentage of killed cells. The cytotoxic activity was carried out before and after lymphocyte ($1x10^6$/ml) incubation with IL-2 (1000 U/ml) for 5 days. Before therapy NK and LAK activity of the patients (5.2 ± 5.4% and 25.4 ± 8.2%) was not different from the controls (8.1 ± 4.0% and 24.5 ± 7.6%), after IL-2 incubation the increase of NK activity was similar in control subjects and patients (from 8.1 to 23.1 ± 7.6% and from 5.1 to 24.0 ± 5.5% respectively). At the end of the therapy both NK and LAK activity were reduced (2.0 ± 1.2% and 14.5 ± 5.1% $p<0.005$) and IL-2 incubation only partially restored the cytotoxic activity (from 2.0 to 17.5 ± 5.1%). These results show that a condition of immunosuppresion occurs in patients who undergo chemotherapy; this condition may make easier the relapse of malignancies and has to be taken in account by planning the strategies for achieving a durable complete remissions.

T 48 ANTIPHOSPHOLIPID ANTIBODIES : PREVALENCE, CLINICAL SIGNIFICANCE AND CORRELATION TO CYTOKINE LEVELS IN NON-HODGKIN'S LYMPHOMA.

A. Perrotti, E. Stipa, F. Oliva, G. Papa et al. Division of Haematology, Ospedale S. Eugenio, Roma

While initially described in sufferers from systemic lupus erythematosus, antiphospholipid antibodies (APA) are increasingly recognized in a broad spectrum of clinical conditions, most importantly in relation to thromboembolic events; occasionally they may also be found in otherwise healthy individuals. Although studies are under way in many countries assessing the related risks of elevated APA in groups of patients with specific diseases, available data on haematological malignancies are still lacking. This study was designed to explore the prevalence and clinical significance of elevated APA titres in patients affected by high-grade non-Hodgkin's lymphoma (NHL). We also analyzed possible correlations with circulating levels of IL-6 (IL-6), and the soluble form of the receptor for interleukin-2 (sIL-2r). Twenty newly-diagnosed patients with NHL were investigated. Tests for APA included the measurement of anticardiolipin antibodies (ACA) with a solid-phase immunoassay, and the detection of the lupus-like anticoagulant (LA) activity. Five patients with NHL (35.7 %) presented elevated APA at diagnosis, as compared to 3 of 174 persons of the control group ($p<0.0001$). APA titres normalized in all patients responding to treatment, whereas non-responders retained elevated levels. In addition, 2 patients who had normal APA at diagnosis and were either refractory to treatment or in relapse, subsequently developed LA and/or ACA positivity. At presentation, the mean levels of IgG- and IgM-ACA in patients were not significantly different from controls, and the correlation between ACA and LA reached just 30 %. With regard to the clinical course, we were not able to detect any statistically significant difference between patients with normal and elevated APA. Pretreatment concentrations of IL-6 and and sIL-2r were found significantly elevated compared to controls ($p=0.009$ and $p=0.024$ respectively). In addition, the levels of these cytokines correlated with IgG-ACA at the different times of laboratory investigations. These results demonstrate that APA may have a role as markers of disease activity and progression in NHL.

T 49 IMMUNOMORPHOLOGICAL AND NUCLEOLAR MARKERS STUDY IN NON-HODGKIN'S LYMPHOMA.

D. Adomaitienė, E. Felinskaitė, G. Griciūtė, G. Kasparičnaitė. Lithuanian Oncology Center, 2600 Vilnius, Lithuania

44 cases of non-Hodgkin's lymphoma (NHL) are reported. The median age at diagnosis for the entire group of patients with NHL was 55 years (range, 8 to 81) and the male to female ratio 1,05 : 1. Immunohistochemical studies using a small panel of 4 monoclonal antibodies were carried out on paraffin-embedded tissues to characterize the neoplastic cells. A routine immunoperoxidase technique was used to identify T-cells (UCHL1; CD45RO), B-cells (L26; CD20), leucocytes (LC; CD45R) and myeloid/histiocytic cells (MAC387) in tumor tissues sections. Determination of lymphoid cell lineage in NHL by immunophenotypic analysis revealed that 61% of all cases were identified as neoplasms of B-cell origin, 22% - as T-cell lymphomas. Remaining cases were not identified; most of them were positive for both cell markers. B- and T-cell lymphomas exhibited the heterogeneity of morphologic characteristics with the prevalent presentation of difuse large-cell lymphomas. Evaluation of immunohistochemical findings displayed that immunomorphological pattern of NHLs in Lithuania seems to be similar to that in other European countries. Identified B- and T-cell lymphomas were processed for argyrophilic nucleolar organizer regions (Ag-NORs) silver staining to reveal markers correlating with proliferative activity of neoplasms. The number of Ag-NORs dots and their morphological characteristics such as distribution pattern, size and shape of the granules were evaluated. Variations in numbers as well as differences in the distribution pattern of Ag-NORs dots were found among different histological subtypes of NHL. These results did not provide evidence for differential activity expression associated with immunophenotypic expression of NHL. The available data demonstrated an increasing trend in numbers of Ag-NORs particles with increasing malignancy. Based on these findings, it is suggested that nucleolar markers may assist in assessing the degree of malignancy.

T 50 INTRATHYMIC DEVELOPMENT AND DIFFERENTIATION OF HUMAN T-CELLS.

E.K. Waller, D. Kraft, and A.S. Majumdar. Division of Oncology, Stanford University Medical Center, Stanford, CA 94305-5468

Normal human T-cell development was studied using immunodeficient SCID mice engrafted with fragments of human fetal thymus (SCID-hu) as a model system. Intrathymic transfer of FACS-sorted and fluorescently labelled thymic progenitors from fetal thymi to heterologous SCID-hu thymic grafts revealed the pathway of normal thymic maturation. $CD3^-4^-8^-$ thymic progenitors rapidly differentiated to a $CD3^-4^+8^-$ intermediate population, then to cells expressing both CD4 and CD8 as well as low levels of CD3. Multiparameter FACS analysis using the DNA-binding dye Hoechst demonstrated that a high fraction of the $CD3^-4^-8^-$, $CD3^-4^+8^-$, and $CD3^+4^+8^+$ populations were in cell cycle. The $CD3^+4^+8^+$ developmental stage corresponds to the point at which T-cells become antigen responsive. We investigated the effects of antigen on human T-cell development by injecting SCID-hu mice with staphylococcal enterotoxin superantigens, and then analyzing the pattern of T-cell receptor (TCR) expression, the developmental stage, and the activation state of thymocytes exposed to superantigens *in vivo*. The most mature T-cell populations, the $CD3^+4^+8^-$ and $CD3^+4^-8^+$ subsets, expressing high levels of the cognate Vβ TCR, were deleted following superantigen exposure. The effect of superantigens on less mature T-cell subsets was different: we found that there was a preferential activation and proliferation of $CD3^+4^+8^+$ cells that expressed low levels of the TCR. We propose a model, based on these results, that activation and proliferation of immature T-cells expressing both CD4 and CD8 as well as low levels of the TCR and, is a consequence of intrathymic antigen exposure.

T 51 MALIGNANT HISTIOCYTOSIS. RECLASSIFICATION BY IMMUNOPHENOTYPIC ANALYSIS.

M.E. Williams, K.M. Sandved, C.E. Hess, L.K. Gross and D.J. Innes. Univ. Virginia School of Medicine, Charlottesville, VA, 22908, USA.

Malignant histiocytosis (MH) is a neoplastic proliferation of cells resembling true histiocytes frequently displaying hemophagocytic activity. Accurate classification is often difficult due to the large number of overlapping benign and malignant entities resembling MH. We utilized a panel of immunophenotypic markers to reassess nine cases originally defined as MH from 1972-89; two cases were diagnosed at autopsy. Each case was morphologically consistent with MH on review, and each showed evidence of hemophagocytosis.

Case	LCA	L26	CD3	UCHL1	LeuM1	Mac387	CD68	S100	Lyso	Kil
1	-	-	-		-	-	+	-	+	-
2	-	-	-	-	-	+	-	+	+	+
3	-	-	-		-	-	+	+	+	-
4	+	-	-		-	-	+	-	-	-
5	+	-	-		-	-	+	+	-	+
6	-	-	-		-	-	-	-	-	+
7	-	-	-		-	-	-	-	-	+
8	-	-	+	+	-	-	-	-	-	-
9	+	+	-		-	-	-	-	-	+

Four separate entities were identified. Cases 1-5 were "true" MH, while cases 6 and 7 were reclassified as Kil-positive anaplastic lymphoma. Case 8 was reclassified as a T cell NHL (CD3P, UCHL1 and Leu 22 +), while case 9 proved to be a T cell rich B cell NHL (kappa +) with atypical reactive histiocytes. MH patients were older (age 43-79 years) than the NHL patients (19-31 years). Six patients had a fulminant course with survival of only 1-4 months; cases 1,3 and 8 responded to combination chemotherapy and survived 1.5-2.5 years. Thus, immunophenotype analysis led to reclassification of 4/9 morphologically diagnosed MH; such analysis should be utilized in all such cases to distinguish true MH from benign and reactive conditions and from T, B and Kil-positive NHL for optimal treatment and prognostic stratification.

T 52 How to Identify the Relationship between Midline Malignant Reticulosis and Malignant Lymphoma

Yirong Huang, M.D., et al
Cancer Institute/Hospital, Chinese Academy of Medicine Sciences,

This study was designed to analize 104 cases admitted and treated in our hospital, from June 1983 to Dec. 1989, with clinical diagnosis of midline malignant reticuloses and pathological diagnosis of malignant lymphoma. Among those patients the male and female ratio was 2.1 : 1; and the median age was 39 years (range 15-75) . As regarding to the initial symptoms, nasal obstruction, epistaxis and profuse nasal discharge consisted of 55.8%; 24 patients (23.1%) had sore throat and mass in the pharynx. The pathological type was classified as peripheral T-cell lymphoma, except thirteen cases with T-cell unconfirmed or B-cell lymphoma. The primary location of the lesion was to the nose in 62.5%, Waldeyer's ring in 27.9% and others in 9.6%. According to Ann Arbor clinical staging there were 35 stage I, 58 stage II and 11 stage IV. 35 patients were complicated with B-symptom. As to the treatment methods, 27 patients were treated by extensive radiotherapy alone, 46 by radiotherapy followed by adjuvant chemotherapy, and 31 by chemotherapy followed by radiotherapy. Among 104 patients 66.4% were CR, 22.1% PR and 11.5% progressive disease. According to actuarial five year survival method there are I' 93.9%, II' 50.6%, IV' 0% and 63% for the whole group.

Discussion: (1) The authors considered the midline milignant reticuloses should not be equal to peripheral T-cell lymphoma, it was because some of those cases were diagnosed as B-cell lynphoma or non-lymphoma patients. (2) Most of the midline malignant reticuloses appeared in nose, when lesions involve the nose only, the prognosis would be better; if the lesions extended to sinus, outside the nose or to lymph node, the therapeutic effect will drop down obviously. It indicated the stage and extent of the lesion may definitely affect patients' prognosis. (3) Patients with B-symptom also indicated bad prognosis, for them more aggressive chemotherapy may be applied to prolongate their survival. (4) The authors think that midline peripheral T-cell lymphoma is a special type of lymphoma, it is not sensitive to both radiotherapy and chemotherapy. We suggested the tumor dose of radiation should be 55-60 Gy. If the primary lesion located in Waldeyer's ring or presented enlarged neck node, the prophylactic radiation of neck nodes should be given. As to the chemotherapy we have tried to use the regimen CDBVP16P, and some encouraged result were seen. (5) The patients who failed to response the treatment were dead within two years, most of them presented extensive involvement, it seems due to dissemination through the blood stream.

T 53 LETHAL MIDLINE GRANULOMA (LMG): CASE REPORT OF AN UNCOMMON TYPE OF NON-HODGKIN-LYMPHOMA IN AN INFANT.

H.J.Plüss and E. Frey, Division of Oncology, University Children's Hospital, CH-8032 Zürich, Switzerland.

Under the name of LMG, a group of lymphoproliferative disorders of sometimes lymphomatous, sometimes granulomatous histology (resembling Wegener's granulomatosis) is subsumed. The lymphomas are located predominantly in the soft palate (in the midline) and usually exhibit T-cell characteristics. Most of the cases in the literature confirm the name of this disease by a fatal course within a few months.

We present a male infant, who was first seen at the age of 4 months with a subcutaneous nodule appearing 10 days after routine vaccination. Because the histology was showing a leukemia- like infiltrate, the boy was thoroughly examined, including the bone marrow, which showed no leukemia, but pancytopenia. The peripheral blood showed only granulocytopenia (of 0.65 *G/l) then. This finding progressed over the next 4 months, and he developed anemia and thrombocytopenia too (Hemoglobin on June 26,1991: 44g/l, PMS o.11, and platelets 56 *G/l). Fanconi-anemia could be excluded at that point.

At 10 months, he developed a rash and an exopthalmus, and one week later, a wainut-sized tumor in the midline of the soft palate was found (on August 8, 1991). The bone marrow now showed 18% of atypical monocytoid cells, and signs of erythrophagocytosis. Histology revealed a pleomorphic high grade NHL with CD3++, CD5+ and CD43++, but CD1,2,4, and 8, and all B-cell markers were negative. The tumor could, therefore, be classified as post-thymic T-NHL. In addition we found multiple bone lesions (in the CTs and X-rays), an enlargement of the right testicule, and a very high LDH (of 4470 U/l) and IL-2 (of 5295).

Despite his severe pancytopenia, treatment (according to the German BFM-NHL-90 for T-NHL) was started , and resulted in complete normalization oft the peripheral counts within 4 weeks, and disappearance of all infiltrative signs. The further course was only complicated by several infections of the upper respiratory tract, but he remained well until the fall of 1992 when he started losing weight and finally started vomiting. An LP on November 23, 1992 (at 2 2/12 years, 15 months after diagnosis) showed leukemic blasts with T-cell markers. He was put on the BFM-ALL-relapse 90 protocol. Its first treatment course resulted in life-threatening speticemia, from which he still has not recovered completely (on Jan.20, 1993). The further course of this lymphoma therefore remains open and could still prove that the name of this uncommon entity cannot yet be changed.

T 54 TRANSFORMATION IN SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES.

SP Mulligan[1], E Matutes [2], J Hewson[1], D Catovsky [2]. Department of Haematology, Concord Hospital [1], Sydney, Australia; Academic Department of Haematology and Cytogenetics, Royal Marsden Hospital [2], Fulham Road, London, U.K.

Richter's syndrome is well described in chronic lymphocytic leukemia (CLL) and a similar transformation to a large cell lymphoma is recognised in follicular lymphoma. We have studied five cases of an aggressive, large cell lymphoma transformation in patients with splenic lymphoma with villous lymphocytes (SLVL), a recently described low-grade B-cell lymphoproliferative disorder of otherwise good prognosis (Mulligan et al, Br. J. Haematol., 78: 206-209; 1991). Two patients had a B-cell derived large cell lymphoma documented arising in peripheral lymph nodes after 6 and 20 years respectively of stable disease and both had favourable responses to CHOP chemotherapy with achievement of complete remission. Another two patients had a similar rapid transformation two years after splenectomy with a progressive clinical course and minimal response to CHOP chemotherapy but without phenotypic documentation of the cell lineage; both died within 6 months. A fifth patient had stable SLVL for 4 years and then developed fevers, weight loss, progressive pancytopenia and increasing splenomegaly. Splenectomy had a good initial result but after 4-6 weeks progressive pancytopenia recurred and bizarre anaplastic large cells were identified in the marrow and documented as CD2+, CD3+, CD5+, CD7+, CD25+ / CD30- T-cells, and diagnosed as an anaplastic T-cell lymphoma. It is likely that this patient represents a new lymphoma rather than a transformation event. These five cases illustrate that a 'Richter's syndrome' may occur in SLVL, a low-grade B-cell leukaemia / lymphoma.

T 55 CASTLEMAN'S DISEASE: report of 7 cases.

E. Orlandi, M.Lazzarino, §M. Paulli, E. Morra, G. Pagnucco, C. Astori, MC. Buonanno, C. Bernasconi. Chair of Hematology, Division of Hematology. §Anatomic Pathology Section. University of Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy.

We report our experience with 7 cases of Castleman's Disease (CD) observed between 1978 and 1991. Clinical data: median age was 50 yrs (range:25-62); M/F was 4/3. Two pts had localized nodal CD. Five pts had a multicentric CD: peripheral lymph nodes were enlarged in 5 pts, the mediastinum in 1 and abdominal lymph nodes in 1; hepatomegaly was present in 3 pts and splenomegaly in 2; 1 pt had ascites, pleural effusion and edema. Bone marrow biopsy showed non specific lymphoid aggregates in 1/7 pts. Four of 5 pts with multicentric CD presented with systemic symptoms: fever in 4, weight loss in 2, arthralgias in 2, weakness in 2. The time interval between the first symptoms and diagnosis varied from 2 to 12 months. Biochemical abnormalities were detected in all pts with multicentric CD: anemia, elevated ESR and hyperglobulinemia were the most common laboratory findings; in 1 pt a low monoclonal IgA λ was also present. Pathology: the histological classification was as follows: hyaline-vascular type 1 pt (with localized CD), plasma cell type 5, intermediate type 1. Evolution: 2 pts (28%) developed a malignancy while on steroid therapy. Of those, one with multicentric CD plasma cell type CD developed a nodular sclerosis Hodgkin's disease 6 months after the diagnosis of CD and is in remission after ABVD therapy. The other pt, with multicentric CD, developed a non Hodgkin's lymphoma (diffuse,large cell) 27 months from presentation and died of resistant lymphoma 7 months later. Two pts with localized CD are alive and well at 8 and 20 months after surgical excision of the nodal mass. Two pts with multicentric CD are alive at 45 and 60 months on steroid therapy, with recurrent clinical manifestation. Another pt died of unrelated cause 50 months after the diagnosis. Conclusion: steroid treatment seems to be inadequate to control clinical manifestations in multicentric CD, although prolonged survival can be observed; moreover, we confirm the need of careful follow-up in multicentric CD pts because of the risk of developing lymphoid malignancies.

T 56 PULMONARY LYMPHOMATOID GRANULOMATOSIS : SEQUENTIAL USE OF SURGERY AND CHEMOTHERAPY.

A.Abbadessa, G.Corazzelli, S.Giordano, R.Muscherà, G.Arcidiacone, G.De Rosa*. Dipartimento Internistica Clinica e Sperimentale,II Ateneo;*Ist. Anat. Patol.,Univ. Federico II,NAPOLI

Lymphomatoid granulomatosis (LG) is a disease with histologic findings of both an inflammatory process and a lymphoid proliferative disease with extranodal involvement primarily of the lung. Its prognosis is very poor and few patients live as long as two years.

The histologic features of pulmonary LG were found by two different pathologists on biopsies from a 68-year-old man presenting with a round irregular lesion (52 x 60 mm) at the apical segment of right inferior lobe. The patient underwent lobar resection. The histologic picture was characterized by an infiltrative process of blood vessels, with proliferating lymphoid cells and pleomorphic cellular population composed of plasmacytes, immunoblasts and epithelioid cells. Pleura and ipsilateral nodes were involved, and the inferior twig of right pulmonary artery was infiltrated. Extensive staging had revealed subdiaphragmatic disease with lombo aortic, iliac and crural adenophaties (PS III; L+ P+ S- H- N+ M-). Blood chemistry was normal, LDH=218 U/L and Ferritin 800 ng/ml. Two weeks after surgery, V M P combination chemotherapy regimen was started (Mitoxantrone 10 mg/mq i.v. day 1, VP-16 80 mg/mq PO days 1-5, Prednimustine 80 mg/mq PO days 1-5) and monthly delivered up to 6 courses. Hematological toxicity was considerable, extra-hematological quite negligible; nadir WBC counts fell between 400 and 1,900 cells/cmm (median, 1,150). Each subsequent course was administered with one week delay; furthermore a 25 % dose reduction for Mitox and VP-16 became necessary in the 2nd and 4th cycle. antimicrobial prophilaxis was supplied in each course. No severe infection occurred. A Complete Remission (CR) was achieved at the end of therapy and two further courses of the same drugs and scheduling brought persisting high values of Ferritin back to normal. The patient is still in CR at 32 mos. from diagnosis.

The histologic findings and therapeutic outcome are described.