ABSTRACTS

POSTER PRESENTATIONS

P 1 SALVAGE THERAPY IN CHILDREN AND ADOLESCENTS WITH HODGKIN`S DISEASE INCORPORATING IEP (ETOPOSIDE/ IFOSFAMIDE/PREDNISONE). A MULTICENTER TRIAL OF THE GERMAN PEDIATRIC STUDY GROUP

I.Hörnig-Franz, G. Schellong, M. Riepenhausen, R. Pötter*
Children`s Hospital and *Clinic for Radiotherapy,
University of Münster, Germany

The German pediatric study group for Hodgkin's disease (HD) initiated a phase II study for salvage therapy (ST) 1986. The aim of this trial was to test the drug combination IEP (ifosfamide 2000 mg/m^2 p.i. 24 h, days 1-5; etoposide 120 mg/m^2 p.i. 60 mins, days 1-5; prednisone 100 mg/m^2 p.o., days 1-5). IEP was combined with ABVD plus COPP in an alternate way. In some pats COPP was replaced by CEP. Most of the pats received 2 courses each of the 3 combinations. Chemotherapy was followed by involved-field irradiation (IFI), using 10-35 Gy. - From April 1986 to July 1991, 44 pats with early progression (2) or 1st relapse (42) of HD were enrolled in this multicenter trial by 20 hospitals. Male/female ratio is 23/21, median age 13.5 yrs (range 6-19 yrs). Median time to failure was 15 ms (range 1-39 ms). Front line therapy had consisted in 31 pats of 2, 4 or 6 courses of OPA or OPA/COMP without procarbazine (PC) and in 13 pats of some modifications containing PC (OPPA or OPPA/COPP) or etoposide (OEPA or OEPA/COPP) in combination with IFI.
Results (Sept. 1992): 3 pats showed progression during ST and deceased later. 41/44 pats achieved CR. 1 pat in 2nd CR developed ANLL (deceased). 7 pats suffered a 2nd relapse (3 deceased). 33 pats remaind in 2nd CR and 37 pats are alive after a median follow-up of 44 ms (14-74 ms), 1 of the pats in 2nd remission developed thyroid cancer and is well after surgery. Kaplan-Meier estimates at 6 yrs are 0.83 (SD 0.05) for survival and 0.68 (SD 0.10) for freedom from 2nd recurrence.
Side effects of IEP: Leukopenia was the most frequently observed side effect followed by alopecia, nausea/vomiting and mild anemia. Severe complications were not observed.
Conclusions: IEP/ABVD/COPP (or CEP) chemotherapy in combination with IFI seems to be an effective ST of moderate toxicity for children an adolescents with 1st relapse of HD. According to clinical impression IEP was the most efficacious part of the treatment. The favorable results might be influenced by the relative mildness of front-line therapy in most of the pats. Nevertheless, children and adolescents with Hodgkin's disease suffering a 1st relapse should be treated with this effective salvage therapy. High dose chemotherapy with ABMT should be reserved for patients with early progression during front-line therapy or with 2nd relapse.

P 2 LOCALIZED CHILDHOOD HODGKIN'S DISEASE : CHEMOTHERAPY (CT) REGIMEN WITH VP16, BLEOMYCIN, VINBLASTIN AND PREDNISONE (VBVP) BEFORE LOW-DOSE RADIATION THERAPY (RT). RESULTS OF THE STUDY BY THE FRENCH SOCIETY OF PEDIATRIC ONCOLOGY (SFOP).

J. Landman-Parker, O. Oberlin, H. Pacquement, R. Capdeville, J.C. Gentet, H. Rubie, A. Ferster, G. Schaison, G. Leverger, J. Lemerle.

The first study of the SFOP demonstrated the effectiveness of 20 grays (Gy) in involved fields after initial CT (ABVD or MOPP/ABVD)(JCO,Vol 10, 1992, 1602). In further attempt to reduce the long term side effects of therapy, in 1990, the SFOP initiated a new study based on CT devoid of both alkylating agents and anthracyclines.

In clinical stages (CS) I and II, all the patients (pts) were given 4 cycles of VBVP : Vinblastin 6 mg/m² days 1 and 8, Bleomycin 10 mg/m² day 1, VP16 100 mg/m² days 1 to 5, Prednisone 40 mg/m² days 1 to 8. Cycles were repeated every 3 weeks. At completion of the forth cycle, clinical evaluation was performed. Good responder pts were given 20 Gy to initially involved areas (+ lombosplenic field for CS IB and IIB). Poor responder pts were given OPPA CT (Vincristine 1,5 mg/m², Procarbazine 100 mg/m² days 1 to 15, Prednisone 60 mg/m², Adriamycin 40 mg/m² days 1 and 14). After new evaluation, good responder pts were given 20 Gy and poor responder were given 40 Gy.

From January 1990 to December 1992, 85 pts below 16 years of age from 20 centers were included. 50 pts were male. 68 have completed their therapy and are evaluable(29 IA, 28 IIA, 4 IB, 17 IIB). After 4 VBVP, 59 pts were good responders to CT : 33 achieved complete remission and 26 > 70 % remission. They were given 20 Gy RT, with boost up to 40 grays for 6 pts. Eight pts were considered as poor responders to VBVP and received one or two OPPA cycles: 2 pts achieved good response and received 20 Gy, 4 received localized boosts up to 40 Gy, and 2 failed to respond. One of them died of progressive disease.
Present median follow-up of the pts is 21 months. No relapse occurred. The 24 month event-free and overall survivals are 97%

These results are encouraging and longer follow-up is needed. Nevertheless those data supports that localized childhood Hodgkin disease can be cured by CT devoid of alkylating agents and anthracyclines.

P 3 STAGE IV HODGKIN'S DISEASE: RESULTS OF THE STUDY OF THE INTERNATIONAL SOCIETY OF PEDIATRIC ONCOLOGY (SIOP)

O.Oberlin, I.Hörnig-Franz, H.Pacquement, V.Vecchi, M.J.Lacombe, H.P.Wagner, J.Lemerle, G.Schellong for the SIOP Hodgkin's disease Study Group.

In 1987, the SIOP started a study for stage IV Hodgkin's disease (HD) in children with several aims: 1. to reproduce at an international level the favourable results of the two German studies observed in 31 patients (pts); 2. to confirm the results of different teams (French, US) who demonstrated that the radiation therapy (RT) can be limited to 20 grays (Gy) in involved fields after effective chemotherapy; 3. to demonstrate the feasibility of such a study at an international level accepting that participating groups may differ in the staging strategies (Italian and French groups had being using clinical staging including lymphangiogram in their national studies since several years; German group strategy was based on selective laparotomy and splenectomy).

The treatment consisted of 2 cycles of OPPA and 4 cycles of COPP followed by RT. Radiation dose to nodes was 20 Gy after complete remission or 36 Gy after incomplete response.12-15 Gy to the initially involved liver, 12 Gy to the lungs and kidneys if initially involved with incomplete regression after the 2 OPPA cycles. Four national groups from Germany, France, Italy and Switzerland participated as well as some hospitals from Austria, Spain and the Netherlands.

Results: Between October 1987 and March 1992, 65 patients entered the procol (28 boys, 37 girls). Median age at diagnosis was 12 years (range 4-15). 47 pts (72 %) had B symptoms. The predominant histological subtype was nodular sclerosis. In 44 pts, only 1 extralymphatic organ was involved: lung (30), bone marrow (6), liver (5), bone (1), kidney (2). 16 pts had 2 or 3 involved organs. 26 pts underwent laparotomy, 18 were splenectomized and all of them showed macroscopic/histological proof of splenic involvement. Overall, the spleen was affected in 37 pts.

At completion of chemotherapy, 50/57 evaluable pts exhibited complete response or more than 75 % regression of the disease; they received 20 Gy to the involved nodes. Only 7 patients were given 36 Gy to one or several nodal areas because of regression < 75 %. 18 patients received lung RT, 2 patients received kidney RT. Only 3 patients showed progression during initial therapy and received alternative chemotherapy.

By January 93, median follow-up of the patients is 33 months (range 9 - 62 months). 6 pts relapsed after 12 - 25 months. The projected 5 year event-free survival is 82 ± 5 %. 3 patients died (1 after early progression of the disease, 1 after relapse, 1 in complete remission when returning to her home country in Yemen. The projected 5 year survival is 94 ± 4 %.

In conclusion: we demonstrated that an international study could reproduce the good results of the previous German study, that OPPA-COPP chemotherapy followed by 20 Gy represents a valid therapeutic approach for stage IV Hodgkin's disease.

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P 4 COMBINATION THERAPY FOR CHILDHOOD HODGKIN' DISEASE (HD) DEPENDING ON RISK FACTORS.

D. Bayzakova, Z. Kamarli, R. Abdyldaev.
Kyrghyz Research Institute of Oncology and Radiology, Bishkek 720064, Kyrghyzstan.

Chemotherapy (CT) and radiation therapy (RT) of children with HD was performed differentially, depending on the rate of risk that included such factors as the biological activity (increase in ESP, ceruloplasmin, fibrinogen, a2-globulin), the presence of intoxication symptoms, the pubertal age, the large tumor mass in the peripheral lymph nodes and mediastinum (more than 5 cm in diameter), the histologic variant. Forty-five HD patients (pts) were under the observation. According to the risk factors pts were divided into three groups:
Group I (4 pts) - IAa - IIAa
Group II (28 pts) -IIAb, IIBb, IIIAa
Group III (13 pts) -IIIAb, IIIBb, IVAb, IVBb
Group I pts were treated with 6 cycles of DOPP regimen. The consolidation and reinduction courses were not carried out. Group II pts received 4 cycles of CT with ADOPP and ACOPP followed by the focal RT (total dose (TD)=30 Gy); the reinduction CT was given for 1.5 year with a 2-week interval. Group III pts were treated with a 6-cycle induction alternating CT with ADOPP and ACVPP regimens followed by the focal RT (TD=30 Gy); the reinduction with ACOPP and DVPP was performed for 2 years with a 2-month interval.
As a result of treatment, 2 Stage IVBb HD pts died of the disease progression, 4 Stage IIBb, IIIBb HD pts developed the relapse during the 1st year of treatment. The response rate was obtained as follows:

Groups of pts	Complete remission	Disease-free survival (5 yrs)	Survival (5 yrs)
I	100%	100%	100%
II	98%	98%	98%
III	92%	88%	85%

Thus, the differentiating approach to the treatment of childhood HD may improve the survival, remission duration and shorten the duration of treatment.

P 5 Long-Term Follow-up of Patients Treated with COMP or LSA_2L_2 Therapy for Childhood Non-Hodgkin's Lymphoma. JR Anderson, RDT Jenkin, JF Wilson, CR Kjeldsberg, R Sposto, RR Chilcote, P Coccia, P Exelby, S Siegel, AT Meadows and GD Hammond for the Childrens Cancer Group, Arcadia, CA 91066-6012, USA.

We analyzed the long-term results of the Childrens Cancer Group study CCG-551, a randomized trial comparing a 4-drug regimen, COMP, to a 10-drug regimen, LSA_2L_2 for the treatment of childhood non-Hodgkin's lymphoma. The initial results have been previously reported (NEJM 308:559, 1983). In particular, we assessed the likelihood of late relapses or deaths without progression after a median of 8 years of follow-up.

A total of 434 patients [pts] were treated, 68 (11 lymphoblastic [LBL], 11 large cell [LCL] and 46 undifferentiated [UDL]) with localized disease [LD] and 366 (168 LBL, 61 LCL and 137 UDL) with disseminated disease (DD).

Event-free survival [EFS] of patients with LD was 83% for COMP and 85% for LSA_2L_2 at 5 years (p=0.85). Two LBL pts relapsed beyond 2 years on study; both were successfully retreated. Results for DD pts depended on histologic subtype: LBL pts did better when treated with LSA_2L_2 (5-year EFS of 66% versus 33% for COMP [$p < 0.0001$]); COMP was better for UDL (5-year EFS of 50% versus 29% for LSA_2L_2 [p=0.016]). Results were similar for LCL (5-year EFS of 50% for COMP versus 43% for LSA_2L_2 [p=0.22]). Five percent of patients died of treatment-related complications while on therapy (primarily infections). Only 4 deaths without progression have been observed late, off-therapy (2 from restrictive lung disease, 1 from an acute asthma attack, 1 from colon cancer). Survival of patients post recurrence was poor, with long-term survival after recurrence estimated at 11% at 5 years.

Long-term follow-up of pts treated on this study demonstrates that treatment success can be expected in 84% of LD pts. For DD pts, treatment success can be expected in 66% of those with LBL and 50% of those with LCL or UDL. Late adverse events to date have been rare.

P 6 14 RELAPSES OUT OF 225 CHILDREN FROM THE SFOP non Hodgkin'S B lymphoma LMB-89: TREATMENT AND SURVIVAL.

D FRAPPAZ, C PATTE, J MICHON, G LEVERGER, H BERHENDT, H RUBIE, Y PEREL, Y BERTRAND, JC GENTET, A THYSS, C BEHAR, O HARTMANN, T PHILIP for the Société Française d'Oncologie pédiatrique.

1/ The experience with LMB 84 randomized protocol (PHILIP et al, Blood, 1993 In press) and further experience with non randomized patients showed a relapse rate of 35/269 (13%). The rescue protocol with CYVE (high dose Cytarabine-etoposide) obtained a CR2 in 7/17 pts versus 5/13 for other rescues. A massive therapy obtained a cure in 6/11 patients in CR2, 0/4 in PR2, 1/5 in resistant disease. Only one of the 15 patients who did not receive massive therapy is alive.

2/ From 07/89 to 11/92. 225 patients from 41 centers were evaluable for survival in the LMB 89 protocol. This protocol included 3 arms (A, B, C): arm B and C are used for patients with advanced disease respectively without and with CNS and/or massive bone marrow invasion. Arm C received prolonged and more aggressive treatment (including 2 courses of CYVE). There was 14/225 (6%) relapses: 10 stage III, 4 stage IV (2 CNS+, 1 minor and 1 massive marrow invasion). Ten had received LMB 89 arm B, and 4 arm C (2 CNS +, 1 massive marrow invasion, 1 resistant to COP). Median delay from diagnosis to relapse was 5 months (3 to 15). Height patients relapsed on and 6 off therapy. The site of relapse was loco-regional (8 cases of which 4 isolated), CNS (6 cases, 2 isolated), marrow (7 cases, 2 isolated).

Rescue protocol included CYVE for all 10 arm B patients: 7 patients were in CR after one (2 pts) or 2 courses (5 patients), and 3 progressed. For arm C, 2 patient were in CR (1 after a MIME-like therapy by VENOMID, 1 after high dose MTX with IV mercaptopurine and radiotherapy, but both had cleared their CSF with intrathecal therapy before chemotherapy), and 2 progressed.

Consolidation: Eleven patients received a massive therapy with bone marrow rescue. Conditioning included BEAM (10 autologous) or TAM (1 allogeneic transplant). Four of 9 grafted in CR remain in CR (1, 12, 16, 33 mths), 5 progressed. One grafted in partial, and one in progressive disease progressed. Overall, 3/4 patients with isolated loco-regional (all off therapy), and 1/2 patient with isolated CNS disease (on therapy) survived. The 3 patients who could not receive massive therapy (for progression) are dead.

3/ CONCLUSION We thus confirm that 1/ Salvage therapy by CYVE obtains a CR in 14/27(51%) patients. For those who received CYVE before their relapse, alternative treatment should be found. 2/ Patients in CR2 may be cured by aggressive management.

P 7 FAMILIAL AGGREGATION OF HEMATOLOGICAL NEOPLASMS AMONG PATIENTS WITH HODGKIN'S DISEASE AND NON-HODGKIN'S LYMPHOMA. O. Shpilberg, M. Modan, B. Modan and B. Ramot. Institute of Hematology, The Chaim Sheba Medical Center, Tel-Hashomer,52621 Israel.

Familial aggregation of hematological neoplasms (HN) was compared in 22 families of patients with Hodgkin's disease (HD) and 57 families of patients with non-Hodgkin's lymphoma (NHL) with two control groups of 36 families with non malignant hematological hematological disorders (NMHD) and 33 families of patients with type II diabetes mellitus (DM). A self administered questionnaire was used requesting a full list of lst and 2nd degree relatives, their vital status, current age or age at death and their chronic diseases. The HD group included 701 relatives in whom 8 (1.1%) were reported to have HN and the NHL group included 1148 relatives in whom 13 (1.1%) reported to have HN. These rates of familial aggregation were significantly higher (p=0.02 and 0.03) than those of the NMHD and DM groups, respectively. The familial aggregation of HN in both study groups was not disease-specific: Only one relative had HD in the HD group, while the other seven had other HN. Three had undefined HN, 2 NHL, 1 multiple myeloma and 1 leukemia. In the NHL group only 2/13 relatives with HN had NHL, while the others had various types of HN: four had undefined leukemia, 2 acute leukemia, 1 multiple myeloma, 1 chronic lymphocytic leukemia and 1 myeloproliferative disorder. These data are consistent with the hypothesis that there is a genetic predisposition to HD and NHL that may be associated with a defect in the pluripotent hematopoietic stem cell.

P 8 MALIGNANT LYMPHOMAS AND EXPOSURE TO CARCINOGENES. ANALYSIS OF 962 CASES OF NON-HODGKIN LYMPHOMAS. A. Corso, C. Astori, E. Morra, A. Livraghi, A. Santagostino, C. Buonanno, G. Castelli, M. Lazzarino, C. Bernasconi. Chair of Hematology, University of Pavia, Division of Hematology, Policlinico S. Matteo IRCCS, Pavia, Italy.

Exposure to mutagenic agents such as organic solvents, herbicides or insecticides have been postulated as possible risk factors for the development of NHL. There are also indications that the exposure to ionizing radiations (Rx) could be a causative factor in an increased prevalence of this type of hematological malignancy. We reviewed, retrospectively, 962 cases of NHL diagnosed at our department and followed consecutively from 1975 to 12/1992. Three groups could be identified with regard to the exposure: group I included 849 pts not exposed to any known mutagenic agent; group II was composed of 84 pts occupationally exposed to chemicals; group III comprised 32 pts previously submitted to Rx for radiodiagnostic examinations or radiotherapy. The age at diagnosis was slightly lower for pts exposed to chemicals (median 53 yrs) than for pts of the I and III groups (median 56 and 58 yrs respectively). In group II 77 pts (92%) were males, a significantly higher incidence than in the other two groups ($p< 0.001$). A variety of occupations was associated with exposure to solvents with prevalence of farmers (52%) and joiners (17%) and the duration of exposure was 3-50 (median 30) yrs. The majority of pts of group III had been submitted to excessive repeated radiodiagnostic examinations for tuberculosis. For these pts the latency time to NHL was 1-51 (median 13) yrs. There were no significant differences in histopathology among the three groups, nor in clinical presentation at onset, except for a higher incidence of bulky disease in pts exposed to chemicals, and an absence of mediastinic involvement in pts exposed to Rx. We did not notice the significant association between the exposure to chemicals and initial supradiaphragmatic location of the lymphoma, reported in the literature. Response to therapy and survival were analyzed for the three major prognostic groups of WF. Among low-grade lymphoma pts group II pts had a worse prognosis than the other two groups. Among the intermediate-grade pts those unexposed had the best survival, whilst for the high-grade pts the prognosis was similar in the three groups. In conclusion: 1) pts exposed to chemicals are most frequently farmers and males, they show at diagnosis a higher incidence of bulky disease and show the worst prognosis among the low-grade NHL; 2) pts exposed to Rx lack mediastinic involvement at diagnosis; 3) among the intermediate grade NHL exposed pts had a worse prognosis than unexposed ones.

P 9

Evidence for increased incidence of non Hodgkin's lymphomas in Burgundy (France) over a 10-year period (1980-1989). CARLI P.M., BOUTRON M.C., MAYNADIE M., CAILLOT D. Registre des Hémopathies Malignes de la Côte d'Or (équipe associée INSERM-DGS) - Laboratoire d'Hématologie, Hôpital du Bocage, 21034 DIJON, FRANCE.

Increasing incidence of NHL has been reported in several areas of the world and it has been often attributed to new AIDS-related lymphoma cases. Nevertheless, little population based data is available for the 1980s period which corresponds to the arrival of the AIDS epidemic.

A Registry of hematopoietic malignancies was created in January 1980. It registers all HM occuring in subjects living in the department of Côte d'Or enabling us to present detailed time trends for all HM in particular NHL.

All NHL, both nodal and extranodal, were considered. Between 1980 and 1989, 380 new cases of NHL were diagnosed in the Côte d'Or in 214 men and 166 women. Cases were classified according to their histological type in three groups, low, intermediate and high grade as defined by the Working Formulation. Age was presented in three groups (< 35, 35-64, >64).

There was an overall 10.9 % (6.5 - 15.0) annual increase in NHL incidence ($p < 0.001$). This significant increase was observed both in men and in women (respectively + 11.2 % ; + 10.5 % ; $p < 0.01$) non dependant of the age group ; although, it tended to be more important in the youngest age group than in all other age groups (+ 19.1 ; $p < 0.05$).The mean incidence rate was lower in rural areas, but the annual increase was more important in these areas than in urban areas (respectively + 19.6 % ; $p < 0.01$ and + 8.1 % ; $p < 0.01$).The urban to rural ratio in incidence was 4.8 in 1980 and decreased progressively to 1.1 in 1989. As for histological type, increase in incidence was statistically significant in all three groups. The most important increase was, however, observed for high grade lymphomas (+ 20.0 p cent ; $p<0.05$). High grade lymphomas were the less common lymphomas until 1984 to become by 1989 the most common together with intermediate grade lymphomas.

In this series, only one case was associated with an HIV infection. These data indicate that although a significant increase in NHL incidence related to the AIDS epidemic might be expected in the near future, there is an independant dramatic trend which started earlier than the AIDS problem. The reasons for the present changes in NHL in the Western World are largely unknown. Such data should prompt aetiological research.

P 10

HIGH PERCENTAGE OF NEWLY DIAGNOSED MALIGNANT LYMPHOMAS IS HIV-ASSOCIATED. A. Dieterle[1], J. Torhorst[1], T. Cerny [1] for the Lymphoma group of the Swiss working group for clinical cancer research (SAKK)[1] and the association of Swiss cancer registries (ASCR)[1]

In January 1991, the SAKK activated a prospective registration study (SAKK 96/90) for all newly diagnosed malignant Lymphomas in the age group from 16 to 65 years seen at the regional SAKK centers. Completeness of the registration was controlled in collaboration with the ASCR. The study aimed on information about the incidence of HIV associated Lymphomas within the SAKK centers and the distribution of Lymphoma type and stage.
As of November 1992, 315 eligible patients were registered, of which full information is available in 310. By crosschecking with the cancer register data it was confirmed, that registration was nearly complete. Within the regions covered by the cancer registries 37% of all eligible Lymphomas were seen at the SAKK centers. In 39 patients no HIV-test was performed. In 271 HIV-tested patients 39 (14%) HIV-associated Lymhomas (32 Non Hodgkin Lymphomas, 7 Hodgkin diesease) occured. Surprisingly, one non eligible patient aged 77 was found to be HIV positive. Evaluation of the distribution of histology and stage is in progress. Updated results will be presented. From this preliminary data we conclude that a high percentage of the newly diagnosed malignant Lymphomas at the SAKK centers within the studied age group is HIV associated.

P 11

OCCURRENCE OF EBV GENOME IN NON-HODGKIN'S LYMPHOMA SUBTYPES: HIGHEST FREQUENCY IN T-CELL LYMPHOMAS

F. d'Amore[1], B.D. Thomasen[2]
[1]Dept. of Haematology, Odense University Hospital, 5000 Odense C, Denmark
[2]Dept. of Pathology, Odense University Hospital, 5000 Odense C, Denmark

Epstein-Barr virus (EBV) is strongly suspected of playing a pathogenetic role in endemic Burkitt's lymphoma and in immunodeficiency-related B-cell malignancies. However, a possible EBV association with non-Hodgkin's lymphoma (NHL) arising in apparently immunocompetent patients has also been suggested. For this reason, an 'in situ' hybridisation (ISH) survey, based on the archival histologic material of a Danish population-based NHL registry (LYFO), was initiated. Cases were screened by non-isotopic ISH for EBV-encoded small nuclear RNA's (EBER) and for abundant immediate early mRNA's (BHLF). So far 135 patients have been screened. They had a median age of 50 yrs (range: 2-91 yrs) and a M/F ratio of 1.8. Sixty-two cases were of B-cell and 66 of T-cell phenotype (B/T ratio = 0.94); 7 were non-B, non-T. Specific histologic subtypes were: centroblastic/centrocytic (CB/CC) follicular (n = 14), mycosis fungoides (MF) (n = 6), CB diffuse (n = 26), peripheral T-cell (PTL) (n = 31, i.e. 26 high-grade and 5 low-grade), lymphoblastic (LB) (n = 33, i.e. 15 B, 16 T, and 2 non-B, non-T), Ki-1 + anaplastic large-cell (ALC) (n = 18, i.e. 5 B, 8 T, and 5 non-B, non-T), other high-grade histologies (n = 7, i.e. 2 B and 5 T). Of the 135 cases, 22 (16.3%) contained EBV genomes (21 EBER-positive, 1 both EBER- and BHLF-positive). Fourteen of the EBV-positive cases were of T-cell and 7 of B-cell phenotype; 1 case was non-B, non-T. The proportion of T-cell lymphomas was significantly higher among EBV-positive than EBV-negative cases (B/T ratio: 0.50 vs 1.06; $p = 0.02$). With regard to T-cell histologic subtypes EBV genomes were found predominantly in high-grade PTL (10 cases). Other EBV-positive T-lymphomas were: 1 case of MF, 1 of angio-immunoblastic lymphadeno-pathy, 1 of Lennert's lymphoma and 1 of ALC type. The latter, together with an additional EBV-positive ALC (non-B, non-T), accounted for 11.1% of all ALC cases. Among B-cell subtypes, LB had the highest frequency of EBV-positivity (20% of cases). Interestingly, no EBV was found in any of the 16 T-cell derived LB lymphomas. Although rare, a scattered EBV-positivity, restricted to a low number of cells, was also seen in follicle centre-cell derived lymphomas (CB/CC 3 cases; CB 1 case). Among the 22 EBV-positive cases, at least 2 distinctive infection patterns were observed: (i) few infected small lymphoid cells, with or without a component of positive histiocyte-like and/or immunoblastic cells, (ii) infection of the vast majority of the neoplastic cell population.

P 12

EPSTEIN-BARR VIRUS DNA IN CASTLEMAN'S DISEASE. B.Borisch, M.Brönniman, F.Delacrétaz, Ch.Meugé-Moraw, H.Müller, J.-O.Gebbers, J.Laissue. Institutes of Pathology, Bern, Lausanne, Frankfurt and Luzern, 3010 Bern, Switzerland

Castleman's disease (CD) is an uncommon lymphoproliferative disorder with an enigmatic pathogenesis and variable biological behavior. Two variants have been described, a hyaline-vascular (HV) and a plasma cell type (PC). A common component of CD are autoimmune phenomena and immunological abnormalities. In some cases of CD monoclonal B-cell populations have occured and other cases have been complicated by malignant lymphoma. In a recent survey, two of eight CD cases were reported to contain Epstein-Barr virus (EBV) specific sequences.

In the present study we have investigated the occurrence of EBV in 19 cases of CD, 9 of the HV-, 3 of the PC-type and 7 of an intermediate type.Paraffin blocs and fresh frozen material (two cases) were collected from the four institutions. All cases were reviewed by four of us, immunohistologically evaluated and searched for EBV by three different methods: immunohistology for the latent membrane protein (LMP), in situ hybridisation with EBER-probes for EBV-specific RNAs, and PCR for both general and subtype-specific sequences of viral DNA.
No LMP-positive cells were found in any of the CDs. EBER-positive cells were present in 18 cases. All samples were subjected to PCR for beta-globin as a test for the availability of DNA and gave positive results. General sequences of EBV, as shown by PCR for gp 220, were detected in twelve cases. All PC and intermediate types of CD were EBV-positive, whereas only 2/9 HC-variant of CD presented with EBV-sequences.
We found more than half of the CD cases of our study to be EBV-positive. The PC and the intermediate variant of CD were more prone to EBV-association than the HV-type. We conclude that CD is an EBV-associated disease. This phenomenon may be related to immune dysfunctions in CD, especially of the B-cell system.

P 13 IN SITU HYBRIDIZATION (ISH) OF HUMAN T-CELL LEUKEMIA VIRUS TYPE 1 (HTLV-1) pX TAX REGION IN PERIPHERAL T-CELL MALIGNANT LYMPHOMAS IN A HTLV-1-ENDEMIC AREA, KAGOSHIMA, IN JAPAN.

K. Hasui, E. Sato, K. Sueyoshi, S. Kitajima, M. Goto and M. Tokunaga.
Dept. of Pathol., Kagoshima Univ. Faculty of Medicine, Kagoshima, Japan.

A definite diagnosis of adult T-cell leukemia/lymphoma (ATLL) is made by documenting the presence of HTLV-1 proviral DNA in the DNA of leukemic or lymphoma cells (*Takatsuki K et al, GANN Monograph on Cancer Reseach 39, 1992*). Tanaka A et al reported that HTLV-1 proviral DNA pX Tax region can act as a kind of oncogene (*Proc Natl Acad Sci USA 87, 1990*). It is well known that the most peripheral T-cell lymphomas in patients with HTLV-1 infection show pleomorphic and/or large anaplastic appearance. However, a direct evidence of HTLV-1 infection in neoplastic cells has been lacking so far. In order to approach this problem, we analyze 112 cases of peripheral T-cell malignant lymphomas (PTMLs) in a HTLV-1-endemic area by means of in situ hybridization (ISH) of HTLV-1, employing a biotin-labeled DNA probe synthesized by polymerase chain reaction of a set of primers SK43 and SK44 for HTLV-1 proviral DNA pX Tax region. The PTMLs comprised 2 chronic lymphocytic leukemias, 75 pleomorphic lymphomas (T-Pleo), 9 anaplastic large cell lymphomas (ALC), 12 T-zone lymphomas (TzML), 4 lymphoepithelioid cell lymphomas (LeL) and 10 angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) type lymphomas according to the updated Kiel classification. The ISH was performed on paraffin sections pretreated by enzyme, acid and heat. In 93 (83.0%) of 112 PTMLs any of lymphoma cells, intermingling lymphocytes, dendritic cells and epithelioid cells showed positive products of ISH-Tax dominantly in their cytoplasm. In the histological subcategorization of T-Pleo and ALC into ATLL subtype, clear cell subtype and others (*Hasui K, Acta Pathol Jpn, 1991 and Path Res Pract 188, 1992*), 44 of 45 cases of the ATLL subtype and 7 of 20 cases of the others showed ISH-Tax-positive lymphoma cells and none of 18 cases of the clear cell type showed positivity in lymphoma cells. In 17 cases of T-Pleo. in which lymphopma cells were ISH-Tax-negative, a small number of ISH-Tax-positive cells were found only in intermingling lymphocytes. In the low-grade malignant PTMLs, including 9 TzMLs, 3 LeLs and 3 AILD type, both of lymphoma cells and dendritic cells/epithelioid cells were ISH-Tax-positive. One of the TzMLs developed into ISH-Tax-positive T-Pleo. Considering the positive figures of this ISH, this ISH detected mRNA of HTLV-1 pX Tax region. In connection with the molecular analysis of HTLV-1 by Yoshida M. et al (*GANN Monograph on Cancer Reseach 39, 1992*), it is considered that this ISH might detect processes of reproduction, maintaining a persistent infection and activation of HTLV-1 proviral DNA. And ATLL may be defined as T-Pleo with an activation of HTLV-1 proviral DNA. Further comparative studies of T-Pleo and low-grade malignant PTMLs with an activation of HTLV-1 proviral DNA will give a clue to see the final alteration(s) of factor(s) exerting on the ATLL developement in patients with HTLV-1 infection.

P 14 HIGH FREQUENCY OF EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN-1 EXPRESSION IN AIDS-RELATED KI-1 (CD30) - POSITIVE ANAPLASTIC LARGE CELL LYMPHOMAS.

A. Carbone, A. Gloghini, R. Volpe, M. Boiocchi, U. Tirelli, and the Italian Cooperative Group on AIDS & Tumors. Centro di Riferimento Oncologico, IRCCS, 33081 Aviano, Italy.

Immunohistochemical detection of Epstein-Barr virus (EBV) - encoded latent membrane protein-1 (LMP-1) was used for identifying EBV-associated $Ki\text{-}1^+$ anaplastic large cell (ALC) lymphomas occurring in patients with (11 cases) or without (29 cases) HIV infection. In addition, 18 representative cases of other AIDS-related lymphomas and 66 Hodgkin's disease (HD) cases, including 14 cases in patients with HIV infection, were investigated. In patients with HIV infection, LMP-1 was more frequently found in $Ki\text{-}1^+$ ALC lymphomas than in other histotypes, although the difference in EBV association between $Ki\text{-}1^+$ ALC and other lymphomas was not significant; moreover, in these patients the percentage of LMP-1 expressing $Ki\text{-}1^+$ ALC lymphomas was significantly higher than that found in patients without HIV infection (72.7% vs 24.1%; $p<0.01$), thus suggesting an etiologic role for EBV in a large proportion of AIDS-related $Ki\text{-}1^+$ ALC lymphomas. Moreover, the frequency of LMP-1 expression in HD cases (71.4% in patients with and 21.1% in patients without HIV infection) was close to that found in $Ki\text{-}1^+$ ALC lymphomas, supporting the view that the higher frequency of EBV association with both entities detected in patients with HIV infection may be AIDS related.

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P 15 Anaplastic large cell lymphoma in adults : a study of 140 cases with comparison to a cohort of 1225 non anaplastic large cell lymphoma included in the same LNH87 protocol.

MF. d'Agay*, J. Brière, E. Lepage, R. Herbrecht, P. Morel, H. Tilly, M. Symann, F. Reyes, P. Gaulard, G. Delsol, J. Diebold, B. Coiffier. reporting for the GELA.*Laboratoire d'Anatomo-Pathologie - Hôpital Saint-Louis, 1 Av. Claude Vellefaux 75010 - Paris. France.

140 cases of anaplastic large cell (ALC) Non Hodgkin's Lymphoma (NHL) were selected on histological and immunological criteria: 100 % of large anaplastic tumoral cells (secondary anaplastic NHL and large cell with anaplastic component were excluded) and expression of the CD 30 antigen in at least 50 % of the tumoral cells. Among them, 21 cases presented a pecular pattern and were called "borderline forms" with Hodgkin's disease.
The study of the phenotype (on paraffin ± frozen section) disclosed B origin in 43 cases (31%), T in 37 cases (26,5%), B and T in 3 cases (2%), true histiocytic in 3 cases (2%) and "null" phenotype in 54 cases (37,5%) ; these latter included the 21 borderline cases.
Comparison with the clinical data of the 1225 non anaplastic large cell patients showed no difference when considering sex ratio (predominance of men : 64 vs 36 %), staging (stade I-II: 41 vs 37% ; stade III-IV: 59 vs 63%), LDH level, performance status, extra-nodal involvement (even in the mediastinum) except for the skin more often affected (10 vs 4,7 %) and the gut less often involved (5,7 vs 16,5 %).
The frequency of B and T phenotype was similar in these ALC patients compared to the non anaplastic large cell cases where the B phenotype was largely predominant (74% B, 6% T, 20% "null").
All theses patients were included in the same therapeutic protocol (LNH87). The complete response (CR) rate was higher in the anaplastic group (80.7% vs 61.4%), $p<10^{-4}$). The 3 years disease free survival (DFS) was 66% without any difference with non anaplastic cohort (3 years DFS=59%). Univariate analysis of survival demonstrated the favorable influence of anaplastic subtype (3 years survival = 76% vs 51%, p =0.04).Multivariate analysis of survival permitted also to define 6 favorable prognostic parameters: anaplastic subtype ($p<10^{-4}$), LDH < 1N ($p<10^{-4}$), PS 0-1 ($p<10^{-4}$), albumine > 30g/l (p=.002), stage I-II (p=.01) and extra-nodal involvement (p=.02). In the anaplastic population, no difference in terms of CR, DFS and survival appeared between B and T subgroups and between the typical anaplastic and the "borderline" cases.
This study demonstrates the favorable influence of anaplastic histology on response to treatment and survival.

P 16 HISTIOCYTIC SARCOMAS. A REPORT OF EIGHT CASES.

A.F. Lauritzen, G. Delsol, N.E. Hansen, J. Ersbøll, T. Horn, K. Hou-Jensen, E. Ralfkiaer. Departments of Pathology and Haematology, Herlev Hospital and Rigshospitalet, University of Copenhagen, Denmark; Laboratorie d'Anatomie Pathologique, CHU Purpan, Touluse, France; and Department of Pathology, Gentofte Hospital, University of Copenhagen, Denmark.

In this study, we described eight histiocytic sarcomas (HS), identified by examination of more than 2000 malignant lymphomas. All of the tumours were high-grade malignancies consisting of markedly pleomorphic large cells with many mitotic figures. Six of the cases showed aggressive courses and died from disease 0.5 to 48 months (mean: 6.5) after diagnosis. The remaining two patients are alive in partial or complete remission 7 and 12 months after diagnosis. Immunophenotypic examination showed positive reactions for macrophage-related antigens and negative reactions for antigens on B-cells, T-cells, myeloid cells, epithelial cells and melanocytes. T-cell receptor and immunoglobulin genes were studied in three cases and were present in a germline configuration. One of the HS resembled Langerhans cells in phenotype and morphology and was classified as a Langerhans cell sarcoma. The remaining HS did not express accessory cell-associated antigens, but more closely resembled "ordinary" tissue macrophages and were positive for lysozyme and CD68 followed in frequency by CD11c, CD4, CD11b, CDw32, PNA and CD13. Oncoprotein p53 was positive in six cases. It is concluded that rare malignancies show features consistent with the derivation from macrophages, including either accesory cells or "ordinary" tissue macrophages. It is possible that p53 is implicated in their pathogenesis and this issue will be an important topic for investigations in the future.

P 17 CUTANEOUS IMMUNOCYTOMAS: A CLINICOPATHOLOGIC STUDY OF 26 CASES.

JU Rijlaarsdam[1], SCJ van der Putte[2], E Berti[3], H Kerl[4], E Rieger[4], J Toonstra[5], ML Geerts[6], CJLM Meijer[7], R Willemze[1], Depts of Dermatology[1] and Pathology[7], Free University, Amsterdam; Depts of Pathology[2] and Dermatology[5], University of Utrecht; Dept of Dermatology, University of Milan[3], Graz[4] and Ghent[5].

Primary cutaneous immunocytomas (PCI) are rare low-grade cutaneous B-cell lymphomas (CBCL) characterized by a proliferation in the skin of lymphoid, lymphoplasmacytoid and/or plasma cells expressing monotypic intracytoplasmatic immunoglobulins. In the present study we compared the clinical and histologic data of 16 PCI and 10 secondary cutaneous immunocytomas (SCI) in order to find out whether PCI have characteristic clinical and histologic features, allowing differentiation from SCI on one hand and other types of CBCL on the other. Our data show that PCI are a distinct type of cutaneous lymphoma charac-terized by 1) the presence of solitary or localized skin lesions, 2) preferential localization on arms and legs, 3) excellent response to local treatment and 4) a favorable prognosis (15/16 alive, no lymphoma related deaths, median follow-up 30 months). Histologically, PCI are characterized by the presence of nodular or diffuse dermal infiltrates with monotypic lymphoplasmacytoid/plasma cells at the periphery of these infiltrates. In contrast with PCI, patients with SCI generally presented with more widespread skin disease; they often had para-proteins and/or auto-immune disorders, and the monotypic tumor cells were found dispersed throughout the dermal infiltrates. The different preferential localization of PCI (extremities) and primary cutaneous follicular center cell lymphomas (head and trunk) suggests that these two main types of CBCL represent separate entities.

P 18 PERIPHERAL T-CELL LYMPHOMA. A CLINICOPATHOLOGICAL STUDY OF 41 CASES AND EVALUATON OF THE PROGNOSTIC SIGNIFICANCE OF THE UPDATED KIEL CLASSIFICATION.

C.Montalban, JM Castrillo, G Obeso, A Gallego, C Bellas, MC Rivas. Hospital Ramon y Cajal, Fundación Jimenez Diaz, Facultad de Ciencias and Hospital de Leganes. Madrid. Spain

Forty-one non-cutaneous peripheral T-cell lymphomas (PTCL) were classified following the updated Kiel classification. Twenty cases belonged to the low-grade group (T-cell chronic lymphocytic leukemia, 3; lymphoepitelioid, 5; angioimmunoblastic, 4; pleomorphic small cell, 8) and 21 to the high grade group (pleomorphic medium and large cell, 11; immunoblastic, 3; large-cell anaplastic Ki-1 positive, 7). Seventy percent showed a CD4+/CD8- phenotype, 39% a defective phenotype and 88% an activation phenotype. Eighty percent had B symptoms, 63% hepatomegaly, 48% splenomegaly and 26% had involvement of more than three lymphoid areas. Bone marrow was infiltrated in 34%, CNS in 4%, lung in 12% and skin in 14.6%. Seventeen percent presented with extranodal disease and 82.8% had stage III/IV disease. Hypergammaglobulinemia was found in 29%, hypercalcemia in 7%, raised LDH serum levels in 58% and HTLV-I antibodies in only one case. Eighteen of the 37 treated patients (48%) achieved a complete remission, but 33% relapsed. Mortality was 59% and actuarial overall survival at 38 months 0.32. In the comparison of the clinical, analytical and immunophenotypic variables and outcome between low and high grade groups, only the average of bone marrow infiltration in the low grade and stage I-II, presence of defective phenotypes and higher Ki67 positivity in the high grade group were significantly different. In the statistical studies, the extranodal presentation and the failure to achieve a complete remission were the only variables that influenced mortality, there were no significant differences in the general features of the low and high grade groups and only minor differences were found in the immunoblastic and angioimmunoblastic subgroups. There were no differences in the actuarial survival between the low and high grade groups, among the subgroups of the Kiel classification, among stages I to IV, between patients with or without B symptoms, with or without defective phenotypes, Ki 67 positivity over or under 60% or among different CD4/CD8 phenotypes. In this study the updated Kiel classification did not separate groups with a prognostic significance.

P 19 CLASSIFICATION OF PRIMARY CUTANEOUS T-CELL LYMPHOMAS OTHER THAN MYCOSIS FUNGOIDES: PROGNOSTIC IMPLICATIONS.

R.Willemze[1], C.J.L.M. Meijer[2], R.C. Beljaards[1], Depts. of Dermatology[1] and Pathology[2], Free University, Amsterdam.

Primary cutaneous T cell lymphomas (CTCL) other than mycosis fungoides and Sezary's syndrome (SS) represent an extremely heterogeneous group, both clinically and histologically. A clinically relevant classification for these lymphomas is not yet available. In the present study, that was aimed to achieve a reproducible and clinically relevant classification a large number of potentially important histologic parameters, including histologic subtype according to the updated Kiel classification, tumor cell size, CD30 expression as well as clinical parameters were investigated on 82 patients with a primary CTCL (non-MF/SS). Multivariate analysis showed as most discriminating parameter CD30 expression on more than 75% of the tumor cells ($p<0.0001$). Estimated 2- and 4-year-survival were 92% and 85% for the CD30-positive group (n=47), and 48% and 25% for the CD30-negative group (n=35), respectively. Within the CD30-negative group significant differences in survival were found between patients classified as pleomorphic, small or medium-sized cell type (n=8) with a 2- and 4-year-survival of 75% and 50%, pleomorphic, large cell type, as defined by the presence of >30% large/blast cells (n=20), with a 2- and 4-year-survival of 50% and 10%, and diffuse blast cell/immunoblastic subtype with a 2-year-survival of 14% (median survival, 9 months). The subdivision of primary CTCL other than MF/SS on the basis of CD30 expression and, for the CD30-negative lymphomas on histologic criteria represents the first clinically (prognostically) relevant classification for this heterogeneous group of primary cutaneous T-cell lymphomas.

P 20 ACTIVATED HELPER T-CELL MYCOSIS FUNGOIDES REVEALED BY HODGKIN'S DISEASE AND ASSOCIATED WITH LYMPHOMATOID PAPULOSIS.

O. REMAN, , E. LORIER, , F. DREYFUS, , B. REMOND, , A. DOMPMARTIN, , X. LEVALTIER, , X. TROUSSARD, , J. C. MANDARD, , M. LESSANA-LEIBOWITCH, , M. LEPORRIER, . CAEN, PARIS, FRANCE.

Mycosis fungoides is a cutaneous T cell lymphoma characterized by marked epidermotropism of cytologically atypical T lymphocytes with convoluted nuclear contours (Sézary cells or Lutzner cells). Skin infiltrating T cells are usually Helper T inducer cell CD2, CD3, CD4. Occasional cases have been reported to be CD8. The Interleukin 2 receptor (CD25) is infrequently found and CD30 is rarely expressed on the malignant cells. Lymphomatoid papulosis is a self-healing papulo-nodular eruption that although clinically benign, has histologic features of malignancy with an infiltration of large atypical cells surrounded by inflammatory cells. The atypical cells have the immunophenotype of activated T cells (CD25, CD30, HLADR). Approximately 10 to 20 % of the cases of lymphomatoid papulosis is associated with a malignant lymphoma, usually T cell cutaneous lymphoma, Hodgkin's disease or lymphoma of other types.

We report here a case of a peculiar form of CD25, CD30 and HLADR positive Mycosis fungoides revealed by Hodgkin's disease and associated with lymphomatoid papulosis. A 31 year-old woman presented in december 1990 with cervical lymph nodes. During the preceding ten years, she had recurrent erythematous papules occuring in crops. Some lesions healed leaving a scar. On examination, cervical, axillary and inguinal lymph nodes were found and biopsy revealed nodular sclerosis Hodgkin's disease. A computed tomography scan showed latero-aortic lymphadenopathy. Polychemotherapy (MOPP, Mechlorethamine, Vincristin, Procarbazine and Prednisone and ABVD : Adriamycin, Bleomycin, Vindesin and Dacarbazin) with mantle and lombo-aortic field irradiation resulted in a complete response. In mars 1991, the patient noticed pruritus, depigmented area of the trunk and a papulonodular cutaneous eruption over the whole body. Some of these nodules developped necrosis and healed within a few weeks. The histology of necrotic nodules showed lymphomatoid papulosis. Biopsy of depigmented area revealed mycosis fungoides. The patient was treated with topical corticosteroïd and mechlorethamine badigeons without efficacy ; and ultra violet light therapy was poorly tolerated. In december 1991, a CT scan of the chest and the abdomen confirms complete remission.The cutaneous papular and nodular lesions however continue to appear and to resolve spontaneously.

This association of lymphomatoid papulosis, cutaneous lymphoma and Hodgkin's disease is unusual : only one observation was found in the literature (N Engl J Med 1992 ; 326 : 1115-22). The immunophenotype of the cutaneous T cell lymphoma and of the lymphomatoid papulosis (CD25, CD30, HLA-DR) are identical and common clonality is confirmed by an unique T cell receptor gene rearrangement (β chain) in both cases. Despite a diffferent histologically appearance, this strenght hypothesis of an occult, anormal T cell clone which can progress to lymphomatoid papulosis and lymphoma.

P 21 INVOLVEMENT OF BCL-1 IN CENTROCYTIC LYMPHOMA.

C.F. de Boer, S.A.J. Loyson., Ph.M. Kluin, G. Peters, J.H.J.M. van Krieken, M.H.H. Kramer, J.C. Kluin-Nelemans, E. Schuuring. Lab. of Pathology, University of Leiden, 2300RC Leiden, The Netherlands.

Centrocytic lymphoma/intermediately differentiated lymphocytic lymphoma (CC/IDL) is a Non-Hodgkin's lymphoma (NHL), presumably derived from follicle mantle B-cells. A translocation t(11;14)(q13;q32) involving the bcl-1 locus, has been described in about half of these lymphomas, but also in some CLL, PLL and myelomas/plasma cell leukemias. The histopathological classification of CC/IDL can be difficult. To distinguish CC/IDL from other NHL's, and to get better insight in the heterogeneity of breakpoints within the 11q13 region, we are evaluating rearrangements in the 11q13 region and the expression of the involved CCND1/PRAD1 gene. We and others cloned the CCND1/PRAD1 gene, that is often overexpressed in (breast, head and neck) cancer with amplification of the 11q13 region. Using Pulsed Field Gel electrophoresis, CCND1/PRAD1 is the gene most proximal to the bcl-1 major breakpoint cluster (bcl-1/MTC) at a distance of 120 kb. Using 4 available probes covering approximately half of this 120 kb region, we detected rearrangements in 10 out of 20 cases of CC/IDL and in 1/22 morphologically similar (low grade) B-NHL, 0/7 cases of chronic B-cell leukemias with small cleaved cells, 0/4 B-PLL and 0/3 plasma cell leukemias. The single case of non-CC/IDL NHL with a rearrangement was a leukemic immunocytoma with circulating cleaved cells, also previously reported as a single case with a bcl1/MTC breakpoint in a series of 44 chronic B-cell leukemias (Blood 1991;77:1560-4). Bcl-1 breakpoints were found over the whole area: 7 within bcl-1/MTC, 2 within a region 24 kb distant from bcl-1/MTC, and 2 cases 2 kb from CCND1/PRAD1. In all cases with an 11q13 breakpoint, comigration with JH sequences proved t(11;14). It has been suggested that in lymphomas with a rearrangement, the CCND1/PRAD1 gene is overexpressed, whereas it is not expressed in lymphomas without a rearrangement. Our first results on a few cell lines show correlation for the t(11;14) and RNA (over)expression. Presently we are studying protein expression of CCND1/PRAD1 using polyclonal antibodies.

P 22 MANTLE CELL LYMPHOMA: CORRELATION OF CLINICAL BEHAVIOR AND HISTOLOGIC PATTERN.

M. A. Rodriguez, W. Pugh, A. Majlis, F. C. Cabanillas. M. D. Anderson Cancer Center, Houston, Texas 77030.

It remains controversial whether mantle cell lymphoma (MCL), alternately called intermediate lymphocytic lymphoma or centrocytic lymphoma, constitutes a discrete disease entity, and, if so, whether it is properly regarded as a lymphoma of low or intermediate clinical grade. We analyzed 41 untreated patients presenting at MDACC with this diagnosis from 1986 to 1992. The histology was reviewed by an expert hematopathologist (W. P.), and the cases were segregated according to growth pattern as follows: 61% diffuse (DIF), 27% Mantle Zone (MZ), and 12% nodular (NOD. Immunohistochemical studies showed 88%of cases were CD5 antigen (+), and 56% lambda light chain (+). Bcl-1 gene configuration was examined in 18 cases. Rearrangement (R) of the gene was present in 42% of DIF, 50% of NOD, and 25% of MZ cases . The clinical characteristics of the patient (pt) group were as follows: median age=54 years; gender=68% male; Ann Arbor stage IV=68%; B symptoms=12%. Disease in extranodal sites (ENS) was very common (85% of pts.). The most frequent ENS were bone marrow (68%) and GI tract (29%). Involvement of ENS was more frequent in the DIF and NOD cases (96% and 80% respectively), compared to MZ (60%).

The treatment response and survival for the patients according to histologic pattern is summarized in the table below.

Histology Pattern	Total no. pts.	No. treated with AdrCT*	%CR to AdrCT*	5 yr. Survival	p. value
Diffuse	25	17	29	52	} 0.96
Nodular	5	4	25	24	} 0.04
Mantle Zone	11	9	77	100	

*AdrCT=Adriamycin-containing Chemotherapy

The clinical behavior of MCL correlates with its histologic pattern. The DIF and NOD patterns had more extensive disease at presentation, poor response to treatment, and poor survival.

P 23 DIFFUSE SMALL B-CELL LYMPHOMAS: PROGNOSTIC VALUE OF MORPHOLOGIC SUBDIVISIONS.

F. Berger, P. Felman, A. Sonnet, Y. Bastion, G.Salles, P.A. Bryon, B. Coiffier. Service d'hématologie, Centre Hospitalier Lyon-Sud, Pierre-Bénite and Hôpital Edouard-Herriot, Lyon, France.

140 patients (pts) with diffuse small B-cell lymphoma (L) referred to our center for diagnosis and/or treatment between 1988 and 1992 were reviewed to define the clinical and biological pictures and the survival associated with each morphologic subtype. Some pts were seen at time of relapse but all initial materials were reviewed. 57 pts had a small lymphocytic or lymphoplasmacytoid (SLL) L (CLL excluded); 17 pts had a polymorphic immunocytoma (PI); 46 pts had a mantle cell L (MCL); and 20 pts had an unclassifiable L mainly because of the presence of characteristics of several or all subtypes. 1 pt with a monocytoid L, 2 pts with a splenic L with villous lymphocytes, and pts with mucosa-associated lymphoid tissue L were excluded from this analysis. Main clinical characteristics of each subtype are presented in the following table.

	SLL	PI	MCL	unclassifiable	p value
N	57	17	46	20	
Older than 60 y	38%	47%	52%	50%	NS
BM involvement	85%	63%	84%	85%	NS
Large spleen	43%	35%	57%	40%	NS
High LDH level	33%	31%	44%	67%	NS
High β2-macroglobulin	51%	60%	52%	67%	NS
CR rate	37%	47%	39%	35%	NS
Median FFP survival (m)	66	26	15	27	.0001
Median survival (m)	120	42	52	83	.005

Adverse prognostic factors for FFP and overall survival were high β2-microglobulin level ($p<.001$), PI or MCL subtypes ($p<.01$), age older than 60 y ($p<.01$), high LDH level ($p<.05$). Stage, localizations, and type of treatment were not associated with survival. This retrospective analysis shows (1) that among lymphoplasmacytoid L pts those with a PI had a poorer outcome; (2) that MCL pts had a poorer outcome than SLL pts; and (3) the most important prognostic factors for these pts whatever the subtype is the β2-macroglobulin level.

P 24 INCIDENCE, PRESENTATION FEATURES AND PROGNOSIS OF LOW-GRADE NON-HODGKIN'S LYMPHOMAS

F. d'Amore[1], on behalf of the Danish Lymphoma Study Group, LYFO
[1] Dept. of Haematology, Odense University Hospital, 5000 Odense C, Denmark

In the period 1.1.83-31.12.88, 1597 newly diagnosed cases of non-Hodgkin's lymphoma (NHL) were included in a Danish population-based NHL registry. Of these, 31% (N=496) were low-grade NHL (LG-NHL) and distributed as follows (Kiel): 9% lymphocytic type (LY), 27% lymphoplasmacytic/-cytoid type (IC), 53% follicular centroblastic/-centrocytic type (CB/CCf) and 11% unclassifiable low-grade.

LG-NHL had an age range of 26-94 years (median: 64 years), an M/F ratio of 0.8 and a stable age-standardised incidence rate of $2.7/10^5$/year. Age-specific IR's showed an exponential rise as a function of age in all subtypes except for CB/CCf, which had a significantly lower median age (61.6). Compared with the intermediate (IG)- and high-grade (HG) group, LG-NHL had more female cases (M/F ratio: 0.79 vs 1.2; $p=0.0002$), a higher frequency of stage III-IV disease (66% vs 53%; $p<0.00005$) and of bone marrow involvement (39% vs 19%; $p<0.00005$). Biochemically, paraproteinaemia was more frequent in LG-NHL than in IG- and HG-NHL ($p<0.00005$), whereas the latter groups had more cases with s-LDH elevation ($p=0.00005$).

A later revision of all IC cases (N=132) distinguished 79 non-polymorphic (ICnp) from 25 polymorphic (ICp) cases; 28 cases were otherwise classified. In 34 LG-NHL patients histologic conversion to a higher malignancy grade was established. The most frequent transformation patterns were: CB/CCf to CB diffuse (22 pts, M/F ratio=0.38) and LY to immunoblastic or CB type (6 pts, M/F ratio=1).

The 7-year survival for LG-NHL was 63% (intermediate: 48%, high-grade: 38%; $p<0.00005$). A Cox-regression analysis identified following adverse factors for cause-specific survival in LG-NHL: age >50 with a relative risk (RR) of 3.2, hepatic involvement (RR=2.1), elevated s-LDH (RR=1.9), B-symptoms (RR=1.8) and IC histology (ICnp+ICp) (RR=1.7). ICp had a lower 7-year survival than ICnp ($p=0.0454$). A univariate analysis performed on young LG-NHL patients (≤50 years), where the large majority of cases (79%) had a CB/CCf histology, identified hyperuricaemia, n.of extranodal sites, hepatic involvement, elevated s-LDH, B-symptoms and splenic involvement as high risk factors.

P 25 THE FOLLICULAR NON HODGKIN'S LYMPHOMAS - 3: PROGNOSTIC FACTORS AND STAGING.

J W Denham, G Vaughan-Hudson, B Vaughan-Hudson, M H Bennett, A M Jelliffe, W R Pratt, and E E Denham, for the British National Lymphoma Investigation

The records of 398 patients with Follicular Non Hodgkin's Lymphoma followed for a minimum of 12 years, who were entered into the British National Lymphoma Investigation Trials between 1974 and 1980, have been reviewed to determine what factors independently influence prognosis and whether more satisfactory alternatives to the Ann Arbor staging system are implementable.

Factors that are patient related (age and sex) disease subtype related (histological classification) and disease stage related (number and distribution of lymph regions involved, marrow involvement, the presence of splenomegaly and constitutional symptoms) were examined to define their independent influence on probability of complete response to therapy, probability of relapse free survival and probability of dying from lymphoma.

Of the patient and disease subtype related variables only increasing age of the patient was found to have an independently significant adverse influence on probability of complete response to treatment, relapse free and cause specific survival. Of the disease stage related variables only increasing number of lymph node regions was found to have a similarly significant adverse influence in all subgroups of patients.

The Ann Arbor staging classification fared poorly, minimally separating relapse free and cause specific survival probabilities in patients with the largest staging groupings III and IV in particular.

Simple classifications based on a simple count of lymph node regions involved and the presence of splenomegaly were far more successful in subdividing the series into subgroups of meaningful size with significantly different probabilities of responding completely to therapy as well as relapse free and cause specific survival expectations.

P 26 RESIDUAL MASSES AFTER TREATMENT OF LYMPHOMA: EFFICACY OF MAGNETIC RESONANCE IMAGING IN PREDICTING RELAPSE.

ME Hill, JD MacVicar, S Milan, J Husband, T Hickish, R McCready, J Mansi and D Cunningham. Lymphoma Unit, Royal Marsden Hospital, Sutton, Surrey UK.

Residual masses evident on CT scanning after treatment of lymphoma are frequently observed. If such a mass contains residual active lymphoma then additional therapy may prevent relapse at this site; if there is no residual disease, additional treatment is unnecessary. Previous studies have demonstrated that plain radiology and CT scanning are unable to differentiate residual disease from lymphoma, and that needle biopsy, erythrocyte sedimentation rate (ESR) and Gallium^{67} single photon emission computed tomography (Ga^{67}SPECT) have variable predictive value. Ga^{67}SPECT has however been regarded by many as the best available investigatoin in this situation, but very few studies have compared its efficacy directly with that magnetic resonance imaging (MRI). MRI has the potential to discriminate between fibrosis and lymphoma since active malignant tissue has been reported to have different signal characteristics to both normal tissue and fibrosis.

We have therefore performed this prospective study comparing the efficacy of ESR, Ga^{67}SPECT and MRI in predicting relapse in this setting. A total of 34 patients have been studied, 21 with Hodgkin's disease and 13 with non-Hodgkin's lymphoma. All had MRI within three months of completing treatment and the majority had Ga^{67}SPECT and ESR during the same period. The ESR was taken as positive for residual lymphoma if the level was >30 mm in the first hour and all MRI and Ga^{67}SPECT images were similarly ascribed as either positive or negative.

During the first year 11 patients relapsed within the area of the residual mass. The MRI had been positive at the time of completion of treatment in 5 of these patients, ESR positive in 4 and Ga^{67}SPECT in 3 (2 of the 11 did not have Ga^{67}SPECT). The number of false positives was 2, 3 and 1 respectively. Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for the three tests were as follows:

	MRI	Ga^{67}SPECT	ESR	
Sensitivity	45	33	36	
Specificty	90	93	84	(all percentages)
NPV	75	68	70	
PPV	71	75	57	

Using the log rank test MRI was found to be the only investigation in which there was a significant difference between the probability of relapse at the site of the residual mass for a positive and negative result ($p=0.013$). This effect was most marked in patients with Hodgkin's disease and with residual masses above the diaphragm. Combining results of investigations did not improve predictive power.

We conclude that MRI is a valuable investigation which is at least as good as, and probably superior to, Ga^{67}SPECT in this setting, capable of providing clinically relevant prognostic information.

P 27 COMPUTED TOMOGRAPHY ASSESSMENT OF RATE OF REGRESSION AND RESIDUAL MEDIASTINAL MASSES IN HODGKIN'S DISEASE.

LF Diehl, G Petroni, TH Wasserman, KD Hopper, S Sagel, A Gottlieb, B Peterson. Cancer and Leukemia Group B, Lebanon, NH, USA 03766

Residual mediastinal masses after treatment of bulky mediastinal Hodgkin's disease are an important treatment problem. The central therapeutic problem is whether a residual mediastinal mass represents residual disease or fibrosis. Chest radiograph studies, gallium studies, computed tomography (CT), magnetic resonance imaging and even biopsy studies have failed to define the meaning of a residual mediastinal mass. Computed tomography demonstrates more detail and is a readily available method to measure an anterior mediastinal mass. In CALGB 8551, 59 eligible patients with Stage IIB, III and IV Hodgkin's disease and bulky mediastinal masses (mass/thorax > .33) were treated in an identical manner. All patients were treated with 6-8 cycles of MVPP (nitrogen mustard, vinblastine, procarbazine, prednisone), followed by 2500 cGy to the mediastinum, followed by 4 more cycles of MVPP at 1.5 times the dose of cycle #6. In 20 patients, CT were performed pre and post treatment enabling us to study the problem of residual mediastinal masses in this advanced stage, bulky mediastinum, identically treated group. A total of 76 CT were performed. Of these 20 patients, 4 have died and 5 have relapsed. Mass size was measured as the largest diameter of the anterior mediastinal mass. A logarithmic transformation of tumor size and least squares method were used to model the rate of regression. Cox's proportional hazard model was used to examine whether the tumor regression rate was associated with time to relapse or survival. Spearman's rank correlation was used to estimate the correlation between rate of regression, initial size and patient age. Rates of regression were not significantly different between relapsing and non relapsing patients. Ninety five percent (18/19, with 1 no post chemotherapy CT) of patients had residual masses after chemotherapy. Only one patient had no residual mass after chemotherapy. Neither tumor regression nor baseline tumor size were found to be significantly ($p > .15$) associated with time to relapse or survival. A negative correlation was detected ($p=.076$) between the initial tumor size and the rate of regression (the larger the initial tumor, the greater the rate of regression). In summary, the data indicate that a larger mediastinal mass regresses more rapidly than a smaller mass; and to date, there is no evidence of an association between rate of regression and time to relapse or death. In patients with bulky mediastinal masses, residual mediastinal masses are almost always present after chemotherapy and they do not seem to predict for relapse or survival.

P 28 THE GALLIUM SCAN (GS) PREDICTS RELAPSE IN PATIENTS WITH HODGKIN'S DISEASE (HD) TREATED WITH COMBINED MODALITY THERAPY.

FB Hagemeister, L Fuller, MA Rodriguez, P McLaughlin, F Swan, JE Romaguera, F Cabanillas. U.T. M.D. Anderson Cancer Center, Houston, Texas 77030.

We have treated 79 evaluable patients with CS I-II and 27 with PS or CS IIIA or B HD with three cycles of NOVP (Novantrone 10mg/m^2 IV d1, Oncovin 1.4 mg/m^2 IV d8, vinblastine 6mg/m^2 IV d1, prednisone 100mg po qd d1-5, given every 21 days) followed by radiotherapy (XT) to the mantle and upper abdomen (stage I-III$_1$) or mantle, abdomen, and pelvis (III$_2$). Staging methods included CT of the chest, abdomen, and pelvis, lymphangiogram, and high-dose GS (8-10 millicuries) with SPECT imaging of selected nodal areas, including the upper torso and primary sites of disease. After three cycles of NOVP were given, and prior to XT, 42 patients had a repeat GS. Two-year (YR) freedom from progression result for stage I-II was 87%, and 2-YR overall survival was 98%; corresponding results for stage III were 80% and 100%, respectively. Prognostic factors for analysis included the presence of a large mediastinal mass (LMM), hilar involvement, B symptoms (SX), a peripheral nodal mass ≥ 10cm, and III$_2$ disease. By univariate analysis, no pretreatment factor was an important predictor of results. However, we also evaluated the potential impact of GS positivity (+) before XT in determining the chance of relapse. For PT with a GS(+), there was a marked decrease of GS uptake after NOVP, but faint residual was still detected. Characteristics of the 42 PT according to GS results are shown:

Feature	PT NO	GS(+) (%)	GS (-) (%)
All	42	10 (24)	32 (76)
Stage I-II	38	10 (26)	28 (74)
No B SX	36	8 (22)	28 (78)
LMM	22	5 (23)	17 (77)

However, by Kaplan-Meier curves, none of the 32 PT with a GS(-) after three NOVP have had progressive disease compared to two of the 10 with GS(+) ($P=.01$). We propose that a GS(+) after treatment with three NOVP, before XT, may predict relapse, even though the CT shows marked improvement, and the PT receives XT to all known sites of disease.

P 29

Gallium (^{67}GA) scanning as an indicator of survival in Hodgkin's Disease.

Joshua DE[1] McLaughlin A[2] Southee A[2]
Gibson J[1] and Kronenberg H[1].
Department of Haematology[1] and Nuclear Medicine[2]
Royal Prince Alfred Hospital, Sydney Australia

Previous studies of the utility of gallium in the management of Hodgkin's disease have either used inferior techniques or have concentrated on the ability to detect disease rather than evaluate the prognostic impact of the study on ultimate patient outcome. Seventy two patients were studied at diagnosis and again after initial treatment using an optimal technique employing 370 MBq of gallium-67 citrate. The sensitivity of the gallium study at staging in detecting active disease was 100%. The sensitivity at restaging was 93% and the specificity 95%. A gallium positive study at restaging was a grave prognostic indicator for the 28 gallium positive patients - 93% either died or had ongoing progressive disease (2 patients had "false positive" uptake). On the other hand, of 44 gallium negative patients, 68% had a lasting complete clinical remission, a further 11% achieved clinical remission following a single relapse, 18% relapsed and had ongoing disease at a mean of 42 months and 1 patient died. The relapse rate was not influenced by the presence of a residual anatomical (mediastinal and abdominal) mass. Gallium scanning is an accurate, non-invasive predictor of residual active disease in Hodgkin's disease and in particular in patients with residual masses it is able to differentiate those with active disease from those whose mass represents only fibrosis or scar.
It is of prognostic significance and of value in determining those patients who should be considered for high dose therapies with stem cell support.

	Restaging with Gallium			
	Gallium Positive		Gallium Negative	
	Stage I, II	Stage III, IV	Stage I, II	Stage III, IV
CR	1	1	27	3
Relapse - CR	-	-	4	-
Relapse - PD	-	-	2	5
PD	12	3	-	2
Death	3	8	1	-

CR = Complete Remission PD = Progressive Disease

P 30

SOMATOSTATIN RECEPTOR SCINTIGRAPHY [SMS] IN THE INITIAL STAGING OF HODGKIN'S DISEASE. P.J. van den Anker-Lugtenburg , E.P. Krenning , H.Y. Oei , C.J.H. Gerrits , S.W.J. Lamberts and B. Löwenberg. Department of Hematology, Dr. Daniel den Hoed Cancer Center, Rotterdam; Departments of Hematology, Nuclear Medicine and Internal Medicine III, Erasmus University and University Hospital, Rotterdam, The Netherlands.

A variety of human neoplasms express somatostatin receptors. Radiolabeled somatostatin analogues can be used to visualize somatostatin receptor-positive tumors in vivo with a gamma-camera. We performed a prospective study comparing somatostatin receptor scintigraphy [SMS] with conventional staging procedures for initial staging of patients with histologically proven Hodgkin's disease. Conventional staging procedures included tomography of chest and abdomen, bone marrow aspiration and histology and sometimes lymphography. 20 consecutive newly diagnosed patients underwent gamma-camera scintigraphy after i.v. injection of the radiolabeled somatostatin analogue, ^{111}In-[DTPA-D-Phe^1]-octreotide. Planar and single photon emission computed tomography [SPECT] images were obtained at 24 and 48 hours after injection. SMS and conventional diagnostic tests were interpreted independently and the results compared. In case of discrepancies additional radiodiagnostic investigations and if possible histological or cytological examinations were performed to verify the diagnosis. 19/20 [95%] of the patients were somatostatin receptor positive. The results of SMS and conventional staging procedures were concordant in 7 patients. In 9 patients SMS revealed lymphoma localizations which were missed at physical examination and conventional imaging methods. In 3 patients lymphoma localizations were missed by SMS. SMS altered Ann Arbor clinical stage in 4 patients. SMS findings raised the clinical stage in 2 cases and the clinical stage was downgraded in 2 cases. These data show that Hodgkin's disease express somatostatin receptors at very high frequency. In 45% of the patients lymphoma localizations, not detected by the conventional imaging methods, were visualized by somatostatin receptor scintigraphy.

P 31

CLINICAL RELEVANCE OF IMMUNOPHENOTYPIC SUB-CLASSIFICATION OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). G. Pagnucco, L. Bellio, L. Vanelli, A. Santagostino, A. Livraghi, A. Canevari, M. Bonfichi, G. Castelli, E. Morra, M. Lazzarino, E. Brusamolino, C. Bernasconi.
Cattedra di Ematologia, Università di Pavia - Divisione di Ematologia, Policlinico S. Matteo IRCCS, 27100 Pavia, Italy.

To determine the significance of the immunophenotypic heterogeneity of B-cell chronic lymphocytic leukemia we prospectively studied 84 consecutive B-CLL patients, observed within a 5 year period (January 1988 to December 1992), with a large panel of monoclonal antibodies detecting B-cell (CD19; CD20; CD21; FMC-7; PCA1), T-cell (CD2, CD3, CD4, CD5, CD8) and HLA-DR, CD10 and CD11c antigens (Ag). Antigen expression was determined by immunofluorescence and flow cytometry using a FACS (EPICS-C) after appropriate forward light scatter gating of total peripheral blood samples. Surface membrane immunoglobulins (SMIg) analysis and mouse rosettes assays (MR) were also performed. Patients were clinically evaluated and staged according to Binet et al (1981). Peripheral blood smears were classified according to FAB criteria (Bennet et al, 1989). All cases had typical clinical features and sustained elevation (>5.0 x 10^9/L) of a population of mature lymphocytes in the peripheral blood, which formed mouse rosettes (MR > 30%) and were HLA-DR/CD19/CD20 positive. This cohort of B-cell CLL patients was classified according to two distinct immunophenotypic classifications. In the first one the cases were divided in three groups based on the expression of CD5 and T antigens: group I (CD5+, T Ag-) (n=51; 61%), group II (CD5+, T Ag+) (n=20; 24%), group III (CD5-, T Ag-) (n=8; 10%). In the second one the cases were divided in three groups based on the expression of CD5 and of one or more B-cell associated antigens (B Ag) CD10, CD11c, PCA1: group A (CD5+, B Ag-) (n=52; 62%), group B (CD5+, B Ag+) (n=22; 26%), group C (CD5-, B Ag+) (n=4; 5%). Groups were compared according to clinical and laboratory features, FAB subtypes, Binet staging, and survival. Group I had a lower leucocyte count (P<0.02) and group B a higher incidence of splenomegaly (P<0.05). Typical CLL morphology (>90% small lymphocytes) was strongly associated with group I and group A immunophenotype (P<0.001), whereas mixed morphology was more frequent in group B and C (P=0.05). Of interest mixed CLL morphology had a higher incidence of splenomegaly (P<0.02), B and C Binet stage (P<0.05) and a higher leucocyte count (P<0.05) than typical CLL morphology. After a median follow-up of 13 mos. (range, 1- 54.7 mos.) no difference in survival rate was observed among the different immunophenotypic groups. We conclude that immunophenotype studies may be clinically useful in defining subgroups of B-CLL with attendant prognostic relevance.

P 32

QUANTITATIVE ANALYSIS OF STATIN EXPRESSION BY FLOW CYTOMETRY (FC) IN NORMAL AND B CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) LYMPHOCYTES.
S.N.Caplan, C.E.Caplan, M.Trudel, E. Wang. Jewish General Hospital & Lady Davis Research Institute, McGill University, Montreal, Canada

Statin is a 57 kDa nuclear protein first identified in growth-arrested fibroblasts and exclusively found in non-replicating (Go-phase) tissues. Using the antistatin monoclonal antibody (MoAb) S44, we have previously identified and localized statin in normal and abnormal lymph nodes, tonsil, peripheral blood lymphocytes (PBL) and rat spleen by immunohistochemistry (Lab Invest 64: 85a). Since conventional techniques (thymidine labelling and DNA analysis) indicate a very low proliferative index in PBL of most pts with CLL, we investigated whether quantitative expression of statin serves as a differential marker of proliferation in CLL. To assess quantitative expression of statin in normal and leukemic PBL, a membrane permeating technique using buffered formaldehyde acetone (BFA) to determine nuclear statin expression by FC was utilized. A monoclonal anti-DNA and a polyclonal anti-IgG antibody served as positive and negative controls for the BFA fixation technique. BFA-fixed cells from U937, a monoblast cell line showed an increase of statin expression as measured by FC from 19% to 47% after 48 hours of serum deprivation. PBL from normals (N=11), B-cell CLL (N=19) and T-cell CLL (N=1) were studied following co-labelling with pan B (CD 19) and pan T (CD 3) MoAbs and the following results obtained:

		% Statin positive cells
Normal	B cells	93.7 ± 4.6
	T cells	68.5 ± 9.2
B-CLL	B cells	51.6 ± 21.5
T-CLL	T cells	15.3

Unlike normals where nearly all B cells expressed statin, B-CLL cell expression was highly variable from patient to patient (14.4-85.6%). In no case did the percent expression overlap with that of normal B cells. Statin expression was not correlated with clinically relevant prognostic variables including stage, previous treatment, lymphocyte count or doubling time, splenomegaly, or time from diagnosis. These data indicate that, unlike normal B cells which virtually all express statin, CLL B cells have a variable but lower expression indicative of an inherent capacity for abnormal proliferation.

P 33 SERUM HUMAN AND VIRAL IL-10 IN PATIENTS WITH NON HODGKIN'S LYMPHOMA.

J.Y. Blay, N. Burdin, F. Rousset, G. Lenoir, P. Biron, T. Philip, J. Banchereau, M. Favrot
Centre Léon Bérard, rue Laënnec 69008 Lyon, France
Schering-Plough, 69100 Dardilly - IARC, 69008 Lyon, France

Serum levels of human and viral (EBV) IL-10 were measured in 184 patients with non HIV-related non Hodgkin's lymphoma (NHL) including 112 patients with active disease, 42 patients in first partial remission (PR), 30 patients in first complete remission (CR), as well as 60 healthy blood donors. IL-10 was detectable in 46 (42%) of the 112 patients with evolutive NHL, 2 of 42 (5%) in first PR, 1 of 30 (3%) in CR and in none of the 60 blood donors. In 13 patients in whom sequential serum IL-10 determinations were performed, IL-10 was detectable in sera collected at diagnosis and/or relapse but not while in partial or complete remission.
IL-10 was identified as vIL-10 and hIL-10 in respectively 22 and 24 of the 46 patients with active NHL. vIL-10 was detected only in patients with detectable anti-EBV antibodies whereas hIL-10 was observed with a similar frequency in EBV seropositive and negative patients. The presence of IL-10 in serum was observed with a similar frequency in all histological subtypes of NHL according to the WF classification as well as in T and B lymphoma. Serum IL-10 was found not correlated to serum LDH or ß2 microglobulin, age, PS or clinical stage. Among intermediate or high grade NHL, patients with detectable IL-10 at diagnosis had a significantly shorter overall ($p = 0.025$) and progression free ($p = 0.030$) survival. Presence of IL-10 was inversely correlated to survival both in adults and children. Serum IL-10 was associated with a particularly poor prognosis among patients with stage IV disease (4 years survival: 0% vs 85% for patients without IL-10, $p = 0.00004$). Multivariate analysis indicated that serum IL-10 was an independent prognosis factor. These results indicate that serum IL-10 is increased in patients with NHL and correlates to the presence of an active disease. The prognostic value of serum IL-10 suggests that these cytokines play a role in disease progression. Ongoing studies are evaluating the cellular source of IL-10 production in these patients.

P 34 CLINICAL RELEVANCE OF SERUM CYTOKINE LEVELS IN NON-HODGKIN'S LYMPHOMAS.

M. Cantonetti, E. Abruzzese, S. Felici, G. Papa, et al. Division of Hematology, Ospedale S. Eugenio, Rome, Italy.

Recent reports have suggested a clinical significance for pretreatment circulating levels of certain cytokines and the soluble forms of membrane antigens in non-Hodgkin's lymphomas (NHL). This prompted us to initiate a systematical investigation of serum levels of many of these molecules in newly-diagnosed patients with NHL. Sixty-eight patients were studied. Their median age was 36 yrs (range 19-60; 39 were males). In all cases histology was revised according to the Working Formulation: 49 were classified as Immunoblastic Lymphomas (H subtype, WF) and 29 as Large Cell Lymphomas (G subtype, WF); 34 patients presented at least one of the systemic B symptoms (fever, weight loss, night sweats). HIV seropositivity was present in 2. The results showed statistically significant higher average levels of interleukin-6 (IL-6), interleukin-8 (IL-8), the soluble form of the receptor for interleukin-2 (sIL-2r) and the soluble transferrin receptor (sTF-r) in NHL patients compared to controls ($p=0.038$, $p=0.025$, $p=0.021$ and $p=0.038$ respectively). sIL-2r levels were found more elevated in Stages III/IV than in Stages I/II ($p=0.013$), whereas IL-6 concentrations were higher in patients presenting B symptoms ($p=0.008$). Significant correlations were found between erythrocyte sedimentation rate (ESR) and IL-6 ($r=709$), and between B2-m and sIL-2r ($r=0.671$). Our results outline the utility of these measurements in the initial staging of of NHL. sIL-2r was found proportional to tumor mass, given the correlation with the clinical stage and B2-m. Elevated plasma levels of IL-6 are significantly associated with the presence of B symptoms and correlate with ERS. Many patients with lymphoma often suffer from fever, weight loss and night sweats. These symptoms resemble, in many ways, a chronic inflammatory disease and may be induced by cytokines such as IL-6. It would be interesting to investigate with a prospective study whether, as reported by Talpaz et al, IL-6 levels at the time of diagnosis are an independent prognostic factor in NHL.

P 35 Immunohistochemical detection of P-glycoprotein drug resistant gene (MDR) and response to chemotherapy in aggressive Non-Hodgkin's Lymphoma.

N. Gad El-Mawla, N. Mokhtar, H.M. Khaled, N. Anwar, M. Mourad, M.R. Hamza, N. El-Bolkainy, and I. Magrath*.

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

The frequency and clinical significance of P-glycoprotein immunoreactivity of MDR gene in 46 previously untreated aggressive NHL patients were analysed. All patients completed a standard BECOP regimen. Paraffin-embedded material was obtained prior to chemotherapy. Sections were tested for reactivity with JSB-1 monoclonal antibody that recognized cell membrane domain using ABC immunoperoxidase technique. Reactivity to antibody was categorized into strong (>50% +ve cells), or moderate (11-50% +ve cells), and negative (≤10% +ve cells). Positivity appeared as a rim of membranous reaction.

A total of 12/46 (26%) of cases were positive for membranous reaction with variable intensities and count among cases, while the remaining 34 cases (74%) were negative for reaction. The pattern and type of reaction did not relate to any specific histopathologic type or grade according to the Working Formulation.

Patients with no complete remission to BECOP regimen (namely those having partial remission, stable disease, and increasing disease) were positive for reaction 5/7, while those showing complete remission to therapy were negative in 30/33. Six patients were excluded due to death from drug toxicity or lost for follow up.

Results showed a test specificity of 71.4% and a sensitivity of 90.9%. This denotes that the predictivity of a negative result is high(93.7%). However, the predictivity of a positive result is lower (62.5%).

P 36 17p ABNORMALITIES IN LYMPHOID MALIGNANCIES: DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS.

C. Schoch, H. Rieder, Ch. Fonatsch, Arbeitsgruppe Tumorcytogenetik, Institut für Humangenetik, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, 2400 Lübeck, Germany

Recently quite a lot of studies have been performed concerning mutations on the molecular level of the p53 gene, which has been mapped to the short arm of chromosome 17, band p13. However, only little is known about cytogenetic abnormalities of 17p in lymphoid malignancies. In routine analyses we found abnormalities of 17p in tumor material of 11 patients with Non-Hodgkin lymphoma (1 Richter-Syndrom (immunoblastic lymphoma emerged from CLL), 1 centroblastic lymphoma emerged from a centroblastic-centrocytic lymphoma, 2 Burkitt's lymphomas) and acute lymphoblastic leukemia (2 Burkitt's type ALL, 1 pre-B-ALL, 1 pre-pre-B-ALL, 1 T-ALL, 2 pre-T-ALLs). No 17p abnormalities were found in low grade lymphomas or chronic leukemias. A strikingly high proportion of Burkitt's lymphomas/leukemias (4/11) with one of the typical translocations involving 8q24 (locus of the c-myc oncogene) showed structural abnormalities of 17p. These cytogenetic data correspond well with molecular genetic findings of a high p53 mutation rate in Burkitt's lymphoma/leukemia and support the hypothesis of cooperation between myc and p53 shown in a mouse cell line. Remarkably we did not find any rearrangement of 17p in the most frequent type of ALL, c-ALL, but in a relatively high percentage of the less widespread T-ALL. It has to be mentioned, that in two of three T-ALL cases the 17p rearrangement was the sole cytogenetic abnormality, whereas all other nine cases showed additional chromosomal aberrations. There is evidence, that p53 mutations occur later in the course of a malignant disease and are associated with progression to a more aggressive form. Concerning the T-ALLs a different role of 17p anomalies and of p53 mutations could be discussed. Abnormalities of chromosome 17 in lymphoma show a poor clinical outcome, the special role of rearrangements involving 17p13 has not been analysed up to now. The diagnostic and prognostic implications of 17p abnormalities in lymphoid malignancies are discussed.

P 37 EXPRESSION AND STRUCTURE OF P53 ANTIONCOGENE IN HODGKIN'S DISEASE (HD): DISSOCIATION BETWEEN IMMUNOHISTOCHEMICAL EVALUATION AND MUTATION DETECTION BY THE HOT TECHNIQUE.

L. Xerri, R. Bouabdallah, C. Derderian, J. Hassoun. INSTITUT PAOLI-CALMETTES, MARSEILLE, FRANCE

Abnormal p53 expression has been extensively reported in a variety of human malignancies such as carcinomas, sarcomas, lymphomas and a few cases of HD. Since normal p53 is undetectable using standard immunocytochemical techniques, overexpression is usually considered as suggestive of genomic mutations. We have investigated the abnormal accumulation of p53 protein in 35 cases of HD using monoclonal antibody PAb 1801 on frozen sections. We found immunocytochemical expression of p53 protein in 11 out of the 35 cases. Positive cases were of mixed cellularity and nodular sclerosing types. The staining was mainly nuclear and was restricted to the Reed-Sternberg cells and their variants.

Analysis of P53 mutations was performed in 9 cases showing positive staining and in 3 negative cases. Exons 5-6, 7 and 8 of the p53 gene were separately amplified by PCR, using genomic DNA extracted from each sample. Amplified products from HD lesions were hybridized with labelled wild-type P53 products amplified from normal thymocytes, and then analyzed by chemical cleavage with the hydroxylamine-osmium tetroxyde technique, which can detect single base pair mismatches.

P53 mutations were identified in none of the 12 HD cases, but were present in the positive controls represented by cell lines. We conclude that (i) P53 overexpression is a common event in HD (ii) immunohistochemical positivity in HD seems not to correlate with the presence of gene mutations. this latter point may be explained either by a phenomenon of disregulated transcription without structural alteration of the P53 gene or by the fact that the percentage of RSC which are supposed to harbour p53 mutations is under the threshold level of sensitivity of our experiments.

P 38 ABNORMAL P53 PROTEIN EXPRESSION IN HODGKIN'S DISEASE.

A.F. Lauritzen, K. Hou-Jensen, E. Ralfkiaer. Departments of Pathology, Herlev Hospital and Rigshospitalet, University of Copenhagen, Denmark.

P53 is an onco-suppressor gene which is located on chromosome 17. Mutations of the p53 gene are closely associated with malignant transformation under "in vitro" conditions and are the most common genetic alteration in human malignancy. Unlike normal p53 protein which is unstable and undetectable by immunohistology, mutated p53 shows a decreased cell turn-over rate and overexpression as compared to the wild type protein. In this study a panel of four anti-p53 antibodies was applied to cryostat sections and rutine specimens of 52 cases of Hodgkin's disease (i.e., 3 lymphocytic predominance, 33 nodular sclerosis, 16 mixed cellularity). The results show that abnormal p53 is present in Hodgkin's- and Reed-Sternberg cells in more than 80% of the cases. It is suggested that mutations of the p53 gene are frequent in Hodgkin's disease and may be implicated in the pathogenesis of this disease.

P 39 CHROMOSOME ANALYSIS OF NON-HODGKIN'S LYMPHOMAS BY FLUORESCENCE IN-SITU HYBRIDIZATION.

D.W. Hammond, B.W. Hancock and M.H. Goyns. Department of Clinical Oncology, Institute for Cancer Studies, University Medical School, Sheffield, S10 2RX, UK.

We have recently completed a cytogenetic survey of a series of 40 non-Hodgkin's lymphomas (NHL) [Hammond et al., 1992, Cancer Genet. Cytogenet., 61, 31-38]. From this study it was apparent that, even with sufficient numbers of good quality metaphases, there were limits to the analysis when conventional cytogenetic banding methods were used. The origins of derivative and marker chromosomes were uncertain, and submicroscopic rearrangements could not be identified. We have therefore adopted the technique of fluorescence in-situ hybridization (FISH) to further analyse our NHL karyotypes. The use of unique sequence probes has allowed the presence of rearrangements to be investigated. A third of our samples exhibited a deletion of the q arm of chromosome 6, which is where the *MYB* proto-oncogene had been localised. We have used the FISH technique to refine the mapping of this gene to 6q23, and have further identified unsuspected alterations of the 6q- chromosomes in one case of NHL by demonstrating duplication of the *MYB* locus. The use of the related FISH technique of chromosome painting has allowed us begin an analysis of the evolution of the malignant cell karyotypes in some the NHL samples. For example, the t(14;18) is the most common anbormality in NHL cells, but in 10% of our cases what appears to be a typical 14q+ derivative chromosome occurs without the reciprocal 18q- derivative and in the presence of 2 normal chromosomes 18. We have used chromosome painting to prove that in some of these cases the translocated material on the 14q+ was from chromosome 18. This implied that these cells originally contained a t(14;18), that the 18q- was lost and that the remaining chromosome 18 was duplicated. This technique has also allowed us to identify a marker chromosome in one NHL case (which appeared to exhibit monosomy of X) as an abnormal X-chromosome that had most of its q and p arms deleted. The application of the FISH technique to the study of NHL cell chromosomes is therefore likely to enable the identification of most chromosomal abnormalities present, and so may reveal the critical events leading to malignant tranformation of the lymphoid cells. This technique can also be applied to studying chromosomes in interphase nuclei, and we have been able to identify differing copy numbers of chromosomes 6 and the X-chromosomes in NHL cell nuclei. The latter approach can be used to estimate the number of malignant cells in a biopsy sample, and could form the basis of a more refined analysis of interphase cytogenetics.

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P 40 ANALYSIS OF THE *P53* GENE, ITS EXPRESSION AND PROTEIN STABILIZATION IN NON-HODGKIN'S LYMPHOMAS.

M.C.M. Finnegan[1], K.A. Lee[1], J.R. Goepel[2], J. Royds[2], B.W. Hancock[1] and M.H. Goyns[1]. [1]Dept. Clinical Oncology, Institute for Cancer Studies and [2]Dept. Pathology, University Medical School, Sheffield, S10 2RX, U.K.

The *P53* tumour suppressor gene is widely regarded as the most important gene in human malignant disease, however, very little is known of the involvement of p53 dysfunction in the evolution of NHL. We were therefore very interested in assessing its involvement in NHL. Although the characteristic chromosome abnormalities that have often accompanied *P53* mutations in other malignancies, such as monosomy of chromosome 17 or breakpoints at 17p13, were not common in NHL, we decided to analyse both immunohistochemical staining of the p53 protein and changes in *P53* gene structure and expression in the same NHL biopsy samples. Three distinct patterns of p53 protein immunohistochemical staining were observed in the NHL samples. The first type was characterized by positive staining of the majority of cells (3/26), the second pattern by the staining of small foci of cells (3/26), and the third by the staining of occasional cells (20/26). The latter was observed both in NHL samples and in the non-malignant reactive nodes. Southern blot analysis of the NHL DNA samples failed to reveal any evidence for rearrangements of the *P53* gene in any of the samples. A PCR strategy based on chemical mismatch cleavage revealed the presence of a mutation in only one sample, and this was associated with the first type of positive staining pattern. Northern blot analysis demonstrated that *P53* mRNA could not be detected in non-malignant tissue, but it was overexpressed in 11/27 NHL samples, however, this did not correlate with positive staining of the p53 protein. As overexpression of the *MDM-2* gene product is thought to stabilize the p53 protein, we carried out a Northern blot analysis of *MDM-2* gene expression, and found that two of the NHL samples exhibited gross overexpression. Both were associated with the two other patients who had exhibited the first type of p53 staining pattern. It is possible that *P53* mutation or *MDM-2* overexpression may have been present in the foci of cells observed in the second type of staining pattern, but as the foci comprised less than 5% of the total number of cells in the biopsy specimen, this would have been difficult to determine. These data suggest that p53 dysfunction might be an important event in the evolution of only a minority of NHL cases, and that there may be different mechanisms which can lead to p53 protein stabilization in these neoplasms.

This work was supported by the Yorkshire Cancer Research Campaign.

P 41 P53 AS A MARKER OF THE MALIGNANT CELL IN HODGKIN'S DISEASE

P.C. Pasman, A. Tiebosch, L. Vrints, F.L. Erdkamp, W.P. Breed, H.C. Schouten. University Hospital Maastricht, Departments of Internal Medicine and Pathology, Catharina Hospital Eindhoven, Maasland Hospital Sittard and University Hospital Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands.

The p53 gene has a tumor suppressor function. The mutated gene encodes for a protein which has a longer half life compared to the normal p53 protein. This enables the detection of the mutated p53 protein by immunohistochemistry. In this study, 53 lymph nodes involved with Hodgkin's disease were stained with DO-1 and CM-1, two antibodies directed against the p53 protein. Samples from all histologic subtypes were available. DO-1 weakly stained 2/14 samples. CM-1 stained 10/25. When preincubated with Target Unmasking Fluid CM-1 stained 51/53 samples positively. One negative sample did not contain any Reed-Sternberg cells. Only Hodgkin and Reed-Sternberg cells stained positive, however negative Hodgkin and Reed-Sternberg cells were seen in the same sample. The intensity of staining varied within the positive cell population. To investigate whether the expression of p53 is cell cycle related, a double staining with antibodies directed against proteins associated with proliferation and CM-1 is being tested. Based on these results, we conclude that the p53 mutated protein is present in a high number of cases with Hodgkin's disease, which is suggestive for an important event in the pathophysiology of the disease. In addition, because of the absence of positive staining in the surrounding lymphocytes, these cells are not likely to be part of the malignant clone.

P 42 MOLECULAR CLONING OF A NOVEL 11q23 BREAKPOINT ASSOCIATED WITH NON-HODGKIN'S LYMPHOMA.

J. M. Meerabux[1], F. E. Cotter[1], L. Kearney[1], D. Nizetic[2] B. Gibbons[1], T. A. Lister[1] and B. D. Young[1]. [1]ICRF Department of Medical Oncology, St Bartholomew's Hospital, London EC1A 7BE (UK). [2]Genome Analysis, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX (UK).

Mediastinal large cell lymphoma with sclerosis (MLCLS) is a rare subtype of high grade non-Hodgkin's lymphoma of B-cell origin. Conventional banded cytogenetic analysis of one example of such a tumour revealed a complex karyoytpe including a t(11;14)(q23;q32) translocation. A cosmid library was constructed from the genomic DNA of this tumour and the derivative clone carrying the rearrangement was isolated. Fluorescence in situ hybridisation confirmed that the cloned region spanned the translocation in this patient. Furthermore, molecular studies have confirmed that the clone includes a partially rearranged VDJ sequence from the immunoglobulin heavy chain gene on chromosome 14 fused to sequence from chromosome 11 band q23. The sequence from chromosome 11 maps proximal to the CD3 gene cluster and is therefore distinct from both the HTRX-1 gene (rearranged in acute leukaemias) and the RCK gene (rearranged in a cell line derived from a centroblastic B-cell lymphoma). Thus, a new lymphoma associated translocation has been cloned and a single copy probe from the breakpoint region on chromosome 11 has been identified. We are investigating the potential role of this translocation in this sub-type of lymphoma.

P 43 DETECTION OF REARRANGEMENTS OF HUMAN T CELL RECEPTOR AND IMMUNOGLOBULIN GENES IN SINGLE CELLS BY PCR.

J. Roth, H. Daus, A. Gause, L. Trümper, M. Pfreundschuh. Dep. Internal Medicine I, University of Saarland, D-6650 Homburg/Saar, Germany

Rearrangement of immunoglobulin (Ig) and T cell receptor (TCR) genes occurs by close juxtaposition of various gene segments that are dispersed over several kb in their germline configuration. Successful rearrangements that serve as markers for clonality can therefore be detected by polymerase chain reaction (PCR) with primers that correspond to sequences within different gene segments.

In order to detect rearrangements of Ig and TCR genes, primers corresponding to the six different families of the variable heavy chain (VH) genes and the IgH joining regions were constructed. Similarly, primers to the variable regions of the TCR-γ gene and the joining regions were made. These primers were shown to detect rearrangements of the corresponding genes in DNA of various lymphoid cell lines and samples from patients with lymphoid neoplasms. Single cells from cell lines and tumour cells of Hodgkin's disease (4 cases, two of nodular sclerosing-, one of mixed cellularity- and one of lymphocyte depleted- subtype) were isolated by micromanipulation from glass slides. Rearrangements were detected in single cells of lymphoid cell lines, demonstrating the sensitivity of this novel technique. No IgH rearrangements were detected in Hodgkin's cells so far. This technique will help to clarify some of the unresolved questions in Hodgkin's disease.

P 44 WHICH IS BETTER, β OR γTCR GENE ANALYSIS TO DETECT CLONALITY IN PERIPHERAL T CELL LYMPHOMA ?

I. Theodorou[1], M. Raphael[4], C. Bigorgne[1], C. Fourcade[4], C. Haïoun[2], M. Divine[2], C. Lahet[1], G. Cochet1, C. Ducos[3], F. Reyes[2], M.P. Lefranc[5], Ph. Gaulard[3], and J.P. Farcet[1]. [1]: Laboratoire d'Immunologie and INSERM U.91, [2]: Service d'Hématologie Clinique, [3]: Département de Pathologie, Hôpital Henri Mondor, 94010 Créteil, France; [4]: Laboratoire d'Hématologie, Hôpital Pitié-Salpétrière, 75013 Paris, France; [5]: Laboratoire d'Immunogénétique Moléculaire, Université Montpellier II, 34095 Montpellier, France.

The analysis of TCR gene rearrangements is critical to discriminate between monoclonal malignant T cells and polyclonal reactive T cells. The reference method, i.e. Soutern blotting (SB), tends to be replaced by gene rearrangement amplification using polymerase chain reaction (PCR) which is more sensitive and faster. In order to determine whether βTCR or γTCR gene is better to be analyzed in peripheral T cell lymphoma (PTCL), we have studied 36 cases using Jγ and Cβ probes and SB. The 36 cases consisted of 31 lymph nodes whose histology was classified according to the Kiel updated nomenclature and 5 spleens from hepatosplenic T cell lymphomas; lymphoblastic and cutaneous T cell lymphomas were excluded. The T cell phenotype was assessed on the presence of one pan T marker (CD2, CD3, CD5, CD7) and the absence of pan B markers (CD19, CD20). According to TCR expression, the series included 20 cases with TCRαβ, 2 cases with TCRγδ, 12 cases with TCR silent, the 2 remaining cases being not interpretable. SB with the Jγ probe showed 23/36 cases with a TCRγ gene rearrangement (63.8%). SB with the Cβ probe could be performed in 28 cases showing 15 rearranged (53.5%). The 15 cases had both β and γTCR rearrangements, 11 cases had both β and γTCR germinal configuration whereas 2 cases were discordant with a γTCR rearrangement and a βTCR germinal configuration. In addition 35 γ alleles (1.58/rearranged case) and 19 β alleles (1.26/rearranged case) were identified. The γ allele configuration indicated that 32 Vγ I and 3 Vγ II subfamily V segments were involved in the recombinative events with 21 J1/J2, 13 JP1/JP2 and 1 JP segments. Therefore in PTCL, the γTCR gene is more frequently rearranged than the βTCR gene with a higher number of rearranged alleles. The PCR strategy for the diagnosis of clonality in PTCL should be based on the analysis of γTCR gene using in first line primers specific for the Vγ I subfamily and the J1/J2, JP1/JP2 segments.

P 45 A NEW NONRADIOACTIVE METHOD TO DETECT CLONALITY IN PRIMARY CUTANEOUS T CELL LYMPHOMAS.

L. Crosti*, M. Bottaro°, A. Biondi^, E. Berti*, R. Caputo* and N. Migone°. * I Clinica Dermatologica Università di Milano, IRCCS; ° Dipartimento di Genetica, Biologia e Clinica Medica e Centro CNR Immunogenetica e Istocompatibilità, Torino; ^ Clinica Pediatrica Università di Milano, Milano , Italia.

DNA hybridization has been foun to be useful in the diagnosis of T cell malignancy by demonstrating the clonal nature of the disease. However Southern-blot analysis suffers from a number of technical disadvantages, including the time necessary to obtain results and the use of radioactivity. We have investigated an alternative approach for assessing clonality in biopsy specimens from patients with cutaneous T cell lymphomas (CTCL). This approach involves the amplification of rearranged gamma T cell receptor genes by the polymerase chain reaction (PCR) and analysis of this product by non denaturing gel electrophoresis. The clonality was detected , in patients with clear bands of rearrangements observed on Southern-blot ,by the use of PCR amplification and acrylamide gel electrophoresis that revealed discrete bands after the gel is stained with ethidium bromide. Moreover we studied 15 patients with inflammatory dermatoses as control and all the specimens revealed a polyclonal pattern appearing as a diffuse smear along the lenght of the gel. Our finding suggest that PCR combined with non denaturing gel electrophoresis may offer a rapid, nonradioactive and sensitive alternative to Southern-blot analysis for the diagnostic evaluation of patients with CTCL.

P 46 MULTICLONALITY AND ALTERED RFLP PATTERNS FOR IMMUNOGLOBULIN AND T-CELL RECEPTOR GENES IN RELAPSING LYMPHOMAS.

Lindh J, Linderholm B, Hagberg H, Sundström C, Roos G. Departments of Oncology and Pathology, Umeå and Uppsala, Sweden

Introduction: Alteration of morphological appearance as well as of clinical behaviour is common in relapsing non-Hodgkin's lymphomas. The aim of this study was to investigate the stability of the genes encoding the immunoglobulin heavy chain and the T cell receptor in during the course of the disease in relapsing non-Hodgkin's lymphomas.

Material and methods: Nineteen patients with relapsed or progressive non-Hodgkin's lymphomas were analysed with respect to alterations of the restriction fragment length polymorphism (RFLP) pattern for Ig heavy chain (IgH) using probes for the Cu and J-region) and for T cell receptor (T-beta,T-gamma chain) genes. DNA was extracted from tumour material taken at different occasions during the course of the disease.

Results: All 19 cases showed clonal rearrangements of the IgH locus. Seven cases (37%) showed simultaneous rearrangement of the genes coding for the T cell receptors in at least one sample. Three or more rearranged bands, indicating more than one malignant clone, were detected in one case at the time of diagnosis but in 5/19 (26%) cases in DNA from samples taken at relapse. Altogether, in 11 out of 19 cases (58%), changes of the IgH rearrangement pattern could be visualised by RFLP. In all these cases except one, the new RFLP pattern included at least one rearranged band from the pattern of the first taken sample. Five out of 7 cases with clonal T cell receptor rearrangements showed new patterns; 2 cases showing altered restriction fragment length, 3 cases appearance/disappearance of non-germline bands. In 4 out of 6 cases with transformed lymphomas clonal changes were observed at time of transformation. Evolution of clones with different RFLP patterns in different compartments were observed in 2 out of 7 studied cases.

Conclusions: The present study illustrates that non-Hodgkin's lymphomas are unstable not only in the IgH genome, but also in the genes coding for T cell receptors. The observations of clonal evolution, multiclonality and different clones in different compartments, offers an explanation to the troublesome situation when treating relapsed lymphomas.

P 47 DETECTION, CHARACTERIZATION AAND CLINICAL USAGE OF CLONAL HEAVY CHAIN IMMUNOGLOBULINE GENE REARR ANGEMENTS IN B- CELL MALIGNANCIES.

B. Linke[1], M. Suttorp[2], N. Schmitz[2], M. Tiemann[3], J. Bertram[1], I. Bolz[1] and M. Kneba[1]. [1] Dept. of Hematology and Oncology, University Clinics, D 3400 Goettingen; [2] Dept. of Hematology and Oncology and [3] Inst. of Pathology, University Clinics, D 2300 Kiel, Germany.

The third complementarity determining region (CDR 3) of the hypervariable domain of heavy chain immunoglobuline chains (V_H-N-(D_H)-N-J_H- junction) has proven to be a highly variable and clone-specific sequence- region of rearranged heavy chain immunoglobuline genes. We used a PCR assay to detect and characterize mono- or polyclonal rearrangements of heavy chain immunoglobuline genes within the CDR 3 region. The CDR 3 regions were amplified using DNA extracted from clinical specimens (bone marrow, peripheral blood and fresh-frozen or paraffin embedded material) by nested PCR with consensus primers directed to conserved gene segments within the variable (V)- and joining (J)-regions. Individual PCR products were sequenced after cloning. The frequency (number of clones with identical sequence/ number of clones totally sequenced per individual PCR) allowed to distinguish between polyclonal and monoclonal B-cell populations.
We studied 20 B-cell neoplasms (10 acute and chronic leukemias and 10 non Hodgkin's lymphomas). In 17/20 of theese (85%), a PCR product was obtained. The sequence analyses demonstrated in 14/20 of the cases (70%) monoclonal VH-N-(DH)-N-JH- junctions. This sequence information allowed the construction of synthetic allele-specific oligonucleotides as highly sensitive diagnostic probes for the detection of lymphoma cells even at clinically uninvolved sites or in remission bone marrow or peripheral blood samples.

P 48 EXPRESSION OF THE RECOMBINASE RAG 1 IN T CELLS LYMPHOMA

by JP Marolleau*,L Imberti#,MF d'Agay*,A Sottini#,P.Brice*,C Gisselbrecht*and D Primi#.

*Institut d'Hematologie Hopital St Louis, Paris 75010 France ;
#Consorzio per le Biotecnologie University of Brescia Italia

The Human recombinase activating genes (RAG1 and RAG2) are necessary for the rearrangement of Ig and T-cell receptor (TcR) genes . RAG1 and RAG2 are expressed early in the ontogeny of B and T lymphoïd cells but not in mature T or B cells . In hematologic malignancies, transcripts for recombinase genes have been found in acute lymphoblastic leukemia of T and B cell type (JC Bories and all Blood 78-1991 vol 8 2053-2061) but are not present in slg+ B cell proliferation .Recently it was published that in Hodgkin disease RAG genes were usualy not transcribed .
We have analyzed by polymerase chain reaction (PCR) the RAG1 mRNA expression in 15 CD3 positiveT cell lymphoma . According to the International Working Formulation 11 were classified as diffuse mixed lymphomas and 4 as large T cell lymphomas . All were CD3 positive,14 were CD4 and one CD8 .Total RNA was prepared from lymph node specimens and cDNA was obtained by reverse transcriptase . Primers used for the analysis of the presence of RAG1 transcripts were : 1.R3' (5' CAACATCTGCCTTCACATCGATCC 3',antisense) and1. R5' (5' CCAAATTGCAGACATCTAACACT 3' ,sense) .Detection of RAG1 amplified products was carried out by Southern blot with specific labeled RAG1 probe .We found the presence of RAG1 specific RNA in one sample at a high level . This TNHL expressed a mature T cell phenotype CD3+,CD4+ . The results of this study indicate that RAG1 is not usually expressed in mature T cell lymphoma except in one case ..This data confirm that RAG expression is a specific marker of T or Bcells maturation . Unfortunetly we were not capable to perform RAG 2 PCR .

P 49 A COMPARATIVE ASSESSMENT OF PROLIFERATING CELL NUCLEAR ANTIGEN , c-myc p62 AND NUCLEOLAR ORGANIZER REGION STAINING IN NON-HODGKIN'S LYMPHOMAS. A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY OF 200 CASES.

P Korkolopoulou, E Patsouris, GA Pangalis, A Tsenga, Ch Kittas. Pathology Department, Asklepeion Hospital, Voula; Haematopathology Section, Department of Pathology, Medical School, University of Athens; Lymphoma Clinic, 1st Department of Internal Medicine, Medical School, University of Athens; Pathology Department, Tzanion Hospital, Piraeus, Greece.

Proliferating cell nuclear antigen (PCNA) is a nuclear protein maximally elevated in the S phase of proliferating and transformed cells. It is recognised by the monoclonal antibody PC-10 in paraffin sections. The nuclear c-myc p62 protein is the main protein product of the c-myc oncogene and is expressed in late Go/early G1 phases. C-myc p62 can be identified by the monoclonal antibody c-myc 1-9E10 working on paraffin sections. Nucleolar organizer regions (NORs) are loops of DNA which possess the RNA genes and are associated with the nucleous. They can be visualized in paraffin sections by means of the argyrophilia of their associated proteins (AgNORs). We have studied 200 cases of non-Hodgkin's lymphomas (NHL) using the monoclonal antibodies PC-10 and c-myc 1-9E10 and the colloid silver nitrate method. A correlation of PC-10, c-myc 1-9E10 and AgNOR scores with histological grade was found ($p < 0.001$). A linear correlation was also identified among these three proliferation-associated indices (PC-10 v/s c-myc 1-9E10 $r=0.551$, PC-10 v/s AgNOR $r=0.746$, c-myc 1-9E10 v/s AgNOR $r=0.529$ - $p < 0.001$). The correlation was stronger between PC-10 and AgNOR scores, although significant discrepancies in individual cases have been observed. There is also good evidence from our study that the proliferative state of the category G NHL (Working Formulation) is significantly higher than that of the rest of intermediate grade lymphomas (D,E,F). Our findings suggest that the combination of the above three cell proliferation indices may provide useful cell kinetic information from paraffin sections in NHL.

P 50 B-CELL LYMPHOPROLIFERATIVE DISORDERS FOLLOWING ORGAN TRANSPLANTATION: CLINICOPATHOLOGICAL, CYTOGENETIC AND VIROLOGIC CHARACTERIZATION.

D. Liebowitz, J. Anastasi, M. Thangavelu, F. Hagos, B. Marcus, L. Swinnen, T. McKeithan, J. W. Vardiman, M. M. LeBeau and O.I Olopade. University of Chicago, Chicago, IL 60637 and Loyola University, Maywood, IL 60153.

An increased incidence of non-Hodgkin's lymphoma is well recognized as a complication of primary as well as secondary immunodeficiency states. We have studied 34 patients (pts) with immunodeficiency after organ transplantation; 17 liver, 8 heart and 5 kidney, 1 liver/kidney and 3 bone marrow. All pts had received cyclosporine, steroids or azathioprine; rejection episodes were treated with solumedrol or OKT-3. There were 23 males and 11 females; the ages ranged from 10 months (mos) to 62 years. Median time to development of lymphoma was 7 mos (range 1-50 mos). Cytogenetic analyses on 15 pts revealed recurring abnormalities in 9 pts (60%). These abnormalities could be classified into 4 groups (gp): gp1 t(8;14) or t(8;22), 3 pts; gp2 +11, 2 pts; gp3 +9, 2 pts and gp4 other translocations involving 14q32 or 22q11 [t(3;22), t(2;14)], 2 pts. Burkitt's translocations and rearrangements involving 14q32 or 22q11 are known recurring abnormalities in B lymphoid neoplasms. Trisomy 11 has been reported as a recurring abnormality in secondary lymphomas. Including our cases, trisomy 9 has now been seen in 4 cases of post transplant lymphomas. By morphology, 12 pts were classified as having polymorphic lymphoid proliferation (PTLD) and 22 had aggressive large cell or high grade lymphoma. Within the PTLD gp, there were 2 cases which were monoclonal by gene rearrangement studies. All pts with cytogenetic abnormalities were morphologically classified as malignant lymphoma. In cases with sufficient material, the pattern of Epstein Barr virus (EBV) gene expression was studied by immunohistochemistry, western immunoblotting and RNA directed PCR. In 13 out of 17 cases studied so far, EBV nuclear antigen 2 (EBNA-2) and latent membrane protein 1 (LMP1) were expressed in the tumor cells. Two of the 4 tumors without evidence of EBV gene expression stained predominantly with T cell markers. Clinical outcome has been extremely poor, however, 4 pts have achieved significant long term survival following treatment. Our studies suggest that these lymphoproliferative disorders are heterogenous and further studies are needed to identify the different subgroups, and develop rational and effective therapeutic approaches.

P 51 DETERMINATION OF THE GROWTH FRACTION IN NON HODGKIN'S LYMPHOMA IN MONOCLONAL ANTIBODY Ki-S5.

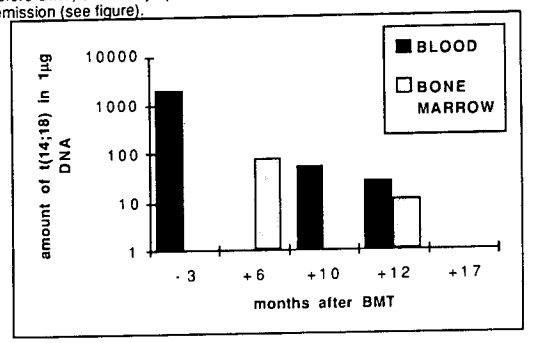
Tiemann, M., Kreipe, H., Wacker, H.-H., Heidebrecht, H.J., Schröter, K., Hauberg, M., Parwaresch, R.
Institute of Pathology and Hematopathology, University of Kiel, Michaelisstrasse 11, D-2300 Kiel, Germany

Relevant prognostic information can be added to the histological classification of non-Hodgkins lymphoma (NHL) by the determination of the growth fraction. In unfixed, fresh tissue samples the Ki-67 antigen has proven its utility as an operational marker of proliferative activity in NHL. We have generated a monoclonal antibody directed against a formalin-resistant epitope of the Ki-67 antigen, designated Ki-S5. The immunoreactivity of Ki-S5 is confined to the nuclei of proliferating cells and unlike Ki-67 no crossreactivity with cytoplasmic antigens of epithelial cells occurs. In 100 cases of different NHL types parallel staining of Ki-67 and Ki-S5 antigen yielded almost identical results in fresh tissues and fixed tissues, respectively ($p<0.00001$; $r=0.95$). The following NHL entities were included: 7 B-CLL, 8 immunocytomas, 22 centroblastic-centrocytic lymphoma, 6 centrocytic lymphomas, 17 centroblastic lymphomas, 9 B-immunoblastic lymphomas, 2 B-lymphoblastic lymphomas, 7 Burkitt's lymphomas, 5 low-malignant T-cell lymphomas of AILD type, 1 lymphoepitheliet lymphoma, 3 T-immunoblastic lymphomas, 5 T-lymphoblastic lymphomas, 8 large cell anaplastic lymphomas. In some cases the Ki-S5 labeling index exceeded that of Ki-67, most probably due to a partial degradation of the epitope recognized by Ki-67 in frozen stored tissue samples. Significant differences in the median Ki-S5 labeling index could be demonstrated between high grade (>50%) and low grade malignant NHL (<30%) although the proliferative activity varied over a considerable range withing identical histological categories ($p<0.001$). We conclude that Ki-S5 provides a reliable means of assessing proliferation in paraffin-embedded NHL specimens. It thus becomes possible to make retrospective studies relating the proliferative activity to the clinical outcome using the archival tissue samples routinely processed.

P 52 QUANTITATION OF FOLLICULAR NON-HODGKIN'S LYMPHOMA CELLS CARRYING T(14;18) IN A PATIENT BEFORE AND AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION.

J. Meijerink, G. Goverde, J. Raemaekers, T. Smetsers, J. Bogman*, T. de Witte, and E. Mensink. Division of Haematology, Department of Medicine, *Department of Pathology, University Hospital St. Radboud Nijmegen, Geert Grooteplein zuid 8, 6500 HB, the Netherlands.

A competitive Polymerase Chain Reaction (PCR) technique was developed (Meijerink et al., Brit. J. Haem., in press) and used to quantify residual malignant cellscarrying t(14;18) in peripheral blood and bone marrow samples of a thirty year old male before and after treatment. In April 1988 a stage IV follicular centroblastic-centrocytic (CB-CC) lymphoma was diagnosed. Doxorubicin-containing chemotherapy induced complete remission (CR), but a relapse was observed in February 1990. Treatment with chlorambucil achieved a CR in October 1990, and the patient then received an allogeneic bone marrow transplantation (BMT) with T-cel depleted marrow from a HLA-identical, MLC non-reactive brother. He is now in continuing complete remission, seventeen months after BMT. Using our assay, malignant lymphoma cells were detected in a lymph node sample in february '90. A blood sample taken three months before BMT, showed lymphoma cells while the patient was in apparent clinical remission (see figure).



In consecutive blood and bone marrow samples after BMT, gradual declining numbers of lymphoma cells were detected. Malignant cells were not detectable anymore seventeen months after BMT. This phenomenon might be explained by graft versus lymphoma activity, although we can not exclude the possibility that the lymphoma cells have lost their proliferating capacity as a consequence of treatment prior to BMT, and slowly disappeared.
These data demonstrate the feasibility of quantitatively monitoring t(14;18) carrying cells in the peripheral blood and bone marrow of follicular CB-CC NHL patients. The number of these cells may reflect the disease activity in the patient.

P 53 ANALYSIS OF THE t(14;18) CHROMOSOMAL TRANSLOCATION USING PCR IN NHL, HODGKIN'S DISEASE AND REACTIVE HYPERPLASIA.

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 t(14;18) chromosomal translocation involving the *bcl*-2 oncogene is demonstrated frequently in patients with low-grade (LG) non-Hodgkin's lymphoma (NHL). It involves the juxtaposition of the *bcl*-2 oncogene on chromosome 18 next to the joining region (Jh) of the immunoglobulin heavy chain gene (IgH) on chromosome 14.

The aim of this study, using the polymerase chain reaction (PCR), was to determine the incidence of the t(14;18) chromosomal translocation (at the major breakpoint region, MBR) in B-cell NHL's (n=75), Hodgkin's disease (n=60) and reactive hyperplasia (n=34). A PCR assay, using a consensus Jh primer in conjunction with a *bcl*-2 primer (targeting the MBR) was performed.

The t(14;18) translocation was detected in 57% (19/33) of LG follicular lymphomas and 21% (9/42) of intermediate/high grade (I/HG) diffuse lymphomas, in 11% (7/60) HD and in 10% (3/34) of reactive lymph nodes. Those HD and reactive samples positive for the translocation had no histological evidence of coexisting low grade NHL.

In 11 NHL patients with known t(14;18) translocation in their tissue biopsies, peripheral blood (PB) and bone marrow (BM) samples at diagnosis were available for analysis. A breakpoint fragment identical to that found in the tissue, was detected in the PB or BM of all 5 LG follicular lymphomas examined, one of which showed histological transformation to an intermediate grade (IG) diffuse lymphoma. The translocation breakpoint fragments were also detected in the PB and BM of all 6 I/HG diffuse NHL's which had no antecedent evidence of LG NHL.

The detection of the translocation in PB and BM of LG follicular lymphomas at diagnosis is not surprising; follicular lymphoma is a systemic disease with an indolent course which does not remit with treatment, in comparison to IG/HG NHL which has a high cure rate.The presence of the translocation in PB and BM of IG/HG diffuse lymphoma may indicate a subgroup of NHL which has evolved from LG NHL, and thus may be less sensitive to treatment.

On the other hand, the detection by PCR of the translocation in ~10% of both HD and reactive lymph nodes may further question the role of this marker as a indicator of malignancy. The increased survival of cells bearing the translocation may predispose to other, as yet, unknown genetic events which render the cells malignant. Transformation of LG follicular to the more aggressive malignant diffuse lymphomas may further our understanding of its oncogenesis.

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P 54 AUTOIMMUNE PHENOMENA IN NON-HODGKIN'S LYMPHOMA

K. Grønbæk, K.G. Schmidt, F. d'Amore
Dept. of Haematology, Odense University Hospital, 5000 Odense, Denmark

There is a well known association between autoimmune diseases and malignant lymphoproliferative disorders. However, previously published data originate from selected patient populations. We studied 626 consecutive cases of non-Hodgkin's lymphoma (NHL) representing all newly diagnosed cases seen at our department between 1.1.83 and 31.8.91. Our department is one of the main collaborating centres in the Danish population-based NHL-registry, LYFO. A detailed analysis of the material showed that it matched the entire LYFO-population as far as age, sex, histology and clinical stage were concerned. This sustains the impression that our patient population is unselected.

Of the 626 patients, 86 (13.7%) had autoimmune phenomena (AP). They consisted of 49 (7.8%) patients with clinical (non-haematological) autoimmune phenomena (CAP) and 37 (5.9%) with immunohaematological phenomena (IHP). IHP included autoimmune haemolytic anemia (15 patients = 2.4%), thrombocytopenia of presumed autoimmune origin (11 patients = 1.8%), Evans' syndrome (4 patients = 0.6%), and a positive direct Coombs' test not associated with other AP (7 patients = 1.1%).

Using a Cox regression model, NHL patients with AP did not differ from other NHL patients regarding survival, time to CR or time to relapse. In the AP group, patients with IHP relapsed earlier than did patients with CAP (median time from CR to relapse: 20 months vs 90+ months; p = 0.03).

Patients in the AP group did not differ from the non-AP patients as regards histology (p = 0.94). In particular, we found very similar frequencies of low-grade histology in these groups (35% vs 33%).

B-cell phenotype was seen in 81% of the AP patients, and in 82% of the non-AP patients. The corresponding frequencies of T-cell phenotype were 14% and 11%, respectively. In the remaining patients the phenotype was unknown. These differences were not significant (p = 0.59)

Our findings indicate that malignant lymphoproliferative diseases are associated with AP more frequently than has previously been suggested, and that -also in contrast with what has been suggested so far- AP are not associated with low-grade histology or B-cell phenotype in particular.

P 55 SERUM SOLUBLE CD23 IS A PROGNOSTIC MARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND STAGE IV LYMPHOCYTIC LYMPHOMA (LL).

D. Bron°, H. Merle Beral+, G. Biron*, A. Leleux°, C. Jacquy°, M. Paesmans°, P. Stryckmans°, C. Fonteyn+, M. Armand+, Y. Delespesse*, L. Binet+ and M. Sarfati*. °Institut J. Bordet, Brussels, Belgium, +Pitié-Salpétrière, Paris, France, and *Hôpital Notre-Dame, Montréal, Canada.

Soluble CD23 (sCD23) is a proteolytic fragment of the type II membrane glycoprotein CD23 antigen. CD23 is overexpressed and abnormally regulated on B-CLL cells and increased sCD23 levels are found in the sera of CLL patients (Blood 71 :94,1988). We prospectively followed 142 CLL and LL patients and collected 902 serum samples that were simultaneously assessed for their content in sCD23. The sCD23 levels ranged from 0.2 to 660 (N 0.2-3)ng/ml with a median value of 54.9 ng/ml and correlated with the clinical stage of the disease at diagnosis. Median survival of CLL patients with sCD23 level >200ng/ml was 46 months whereas survival of patients with ≤200ng/ml was 71 months (p=<0.0001). In a Cox multivariate regression analysis, sCD23 and clinical stages appeared to have a dependent prognostic significance. For 97 CLL patients, we collected 3 or more consecutive samples (n=765) during a 3 to 90 months period with a median follow-up of 40 months. Although sCD23 level was poorly correlated with the lymphocytosis and the serum ß2 microglobulin level, it was strongly correlated (r=0.82) with the Jaksic (√lymphocytosis + height(cm) of the spleen below costal margin + diameter(cm) of the largest lymphnode.). In individual patients, improved clinical status was associated with decrease in serum sCD23 levels whereas disease progression was associated with increased levels. In conclusion, sCD23 appears to be a specific and unique marker that can be used reliably to monitor CLL therapy and most importantly to identify a poor prognostic group of patients who might benefit from more aggressive therapeutic approach.

P 56 SOLUBLE CD23, A MARKER IN CLL and B - CELL LYMPHOMAS REFLECTING DISEASE ACTIVITY AND TUMOR MASS

WW Reinisch, MF Hilgarth, R Mader, V Edward, JD Schwarzmeier. Med. Clinic I, Dept. Haematology, Univ. of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria

CD23 generally known as the low-affinity receptor for IgE (FceRII) is expressed on the surface of B-lymphocytes at the intermediate stage of differentiation and on B-CLL cells. Its soluble form, sCD23 (IgE-BF) has potent BCGF-activity. Recently, it has been shown, that sCD23 is significantly elevated in sera of B-CLL patients. The clinical implications of this finding, however, are still unclear. To obtain further information on the role of sCD23 and the in vivo mechanism of its regulation, we investigated 40 cases of B-CLL with RAI stages O-IV. In addition to the measurement of sCD23 serum levels, we examined the expression of CD23 on MNCs in 17 cases. The results indicate that in all patients with B-CLL sCD23 was highly elevated (median 4544, range 284-20200) as compared to normal individuals (median 113, range 27-1504) and other lymphoproliferative disorders (HCL, T CLL, HL, low grade/high grade (lg/hg) NHL, ALL, MM). Only within the group of lg NHL of B-cell phenotype were similar serum levels to B-CLL measured. Serum concentrations of sCD23 correlated with disease activity as evaluated by RAI stage, lymphocyte doubling time and distinction between active and indolent forms of B-CLL, but not with absolute lymphocyte counts. The response induced by chemotherapy was reflected by a decrease of sCD23 serum levels. The FACS analysis revealed that 75% of MNC in B-CLL were CD23 positive. The CD23 antigen was located independently of stage on the malignant CD19/CD5 positive population. While in lymphomas the CD23 antigen was restricted to the CD19/CD5 positive population, it was found only on CD19 positive cells in healthy donors. Since sCD23 serum levels were only weakly correlated to the absolute numbers of CD23+ MNC in peripheral blood and neither related to the density of CD23 molecules on the cell surface of MNC nor to the product of these two parameters, sCD23 seems to reflect the tumor mass rather than the number of leukaemic cells.

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P 57 PROGNOSTIC IMPLICATIONS OF OCCULT DISEASE IN THE PERIPHERAL BLOOD OR BONE MARROW AT DIAGNOSIS IN NON HODGKINS LYMPHOMA.

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

Non Hodgkin's lymphomas (NHL) are monoclonal, each with a unique and identical rearrangement of either an immunoglobulin or T-cell receptor gene in every malignant cell. Using Southern blot analysis it is possible to detect the presence of 1% of these malignant cells in a tissue sample thereby identifying occult minimal volume disease (MVD). We have prospectively evaluated Southern blot analysis in the detection of MVD as a prognostic marker in newly diagnosed patients with NHL. Approximately 0.1-10ml of bone marrow (BM) and 50ml of peripheral blood (PB) were obtained from 55 newly diagnosed NHL patients during routine pretreatment staging procedures between 1990-1991. DNA was extracted from the mononuclear fraction of BM (n=48) and PB (n=44) samples and digested using the restriction enzymes EcoR1, Hind3 and BamH1. Southern blot analysis was carried out and gene rearrangements were detected by hybridising with both an immunoglobulin heavy chain (JH) probe and T-cell receptor constant region (Tβ) probe to detect clonality in B and T-cell lymphomas respectively. The results of these studies were compared with routine histopathology. There was insufficient DNA for analysis in 22/92 samples. While malignant tumour cells were identified pathologically in 14% (12/70) of specimens, in contrast, gene rearrangements were detected in 43% (30/70). Patients with and without detectable MVD had similar pre-treatment prognostic characteristics. There was no difference in response rate or survival among patients with or without detectable MVD in their BM. However patients (15/35) with detectable rearrangements in their PB had a significantly ($p<0.05$) inferior complete response rate of 47% versus 70%, and had an inferior disease free survival at 2 years, 20% versus 40%, when compared to patients (20/35) without evidence of MVD in their PB. These results indicate that Southern blotting in NHL is superior to standard histopathology in detecting MVD in PB and BM samples. The detection of MVD in the PB, as suggested by these results may have prognostic significance in NHL. In this study the detection of MVD in the bone marrow does not appear to be of prognostic significance. Further studies are indicated to clarify the value of gene rearrangement analysis as a prognostic marker, and its role in the early detection of residual or recurrent disease in patients with NHL, thereby allowing the identification of those patients who would benefit from more intensive therapy including bone marrow transplantation.

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P 58 PROGNOSTIC FACTORS FOR SURVIVAL IN 114 PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA.

S. Cortelazzo,* G. Capnist, * T.Chisesi, A. Rossi, P. Viero, ° V. Rizzoli, ^E. Damasio, # A. Perna, T. Barbui. Divisions of Hematology, Ospedali Riuniti di Bergamo,*Ospedale S. Bortolo, Vicenza,^University of Parma,^Ospedale S. Martino di Genova,#Istituto'M. Negri', Bergamo, Italy.

Waldenstrom's macroglobulinemia is a generalized chronic lymphoproliferative disorder characterized by the production of a monoclonal IgM by well-differentiated plasmocytic lymphocytes. Most patients respond to first-line chemotherapy at varying extent and survival seems to be unaffected by treatment. New drugs such as Fludarabine and 2-CDA are now reported to be active in this disease so, in order to evaluate their role, it would be desirable to identify patient categories at different prognostic risk. With this aim we have retrospectively analyzed a cohort of patients collected in four italian institutions (Bergamo,Vicenza, Genova, Parma).
Between 1976 and 1992 the diagnosis of Waldenstrom's macroglobulinemia was confirmed in 114 pts, 76 males and 38 females, median age 67 (range, 35-87) by the presence of an unequivocal infiltration ≥ 30% of lymphocytes/ lymphoplasmocytes in bone marrow biopsy and a monoclonal IgM ≥ 0.5 g/dL on electrophoresis. Twenty percent of pts had hepato-, 19 % spleno- and 18 % lympho-adenomegaly. Eighty pts received chemotherapy (73% mono and 27% polichemotherapy) while 28 pts remained untreated. Median survival was 108 months (range, 4-147 months).Thirty three pts (29%) died of disease or intercurrent medical illness. Twelve clinico-pathologic parameters at presentation were examined in univariate analysis: age ≤ 65 vs > 65; sex M vs F; IgM ≤ 2.5 g/dL vs >2.5 g/dL; kappa vs lambda ; albumin ≤ 3g/dL vs > 3 g/dL; absence vs presence of cryoglobulins ; Hb ≤ 10 G/dL vs > 10 g/dL; white bood cell ≤ 1.5 x 10^9/L vs > 1.5 x 10^9/L ; platelets ≤ 100 x 10^9/L vs > 100 x 10^9/L; presence vs absence of splenomegaly, hepatomegaly, lymphoadenomegaly. In univariate analysis 5 criteria were found to predict survival: 1) age at diagnosis > 65 years (longrank= 11.7, p >0.01); 2) IgM > 2.5 g/dL(longrank= 11.6, p <0.001); 3) Hb < 10 g/dL (longrank= 11.4, p < 0.001); 4) white blood cells ≤ 1.5 x 10^9/L (longrank= 13.5, p < 0.001); 5) platelets ≤ 100 x 10^9/L (longrank= 13.5, p <0.001).
Using Cox proportional hazard model only 3 risk factors among the 5 were independently of prognostic significance: 1) Age >65 years (p= 0.03); 2) platelets ≤ 100 x 10^9/L (p= 0.05); 3) IgM > 2.5 g/dL (p=0.005).

Thus, in this unselected series 22 % of pts were considered at low risk (i.e. without any of the three afore-mentioned risk factors) and had a 10 years actuarial survival of 88%, while in the high risk group (with all risk factors), only 38 % of pts were projected to be alive at 10 years and the projected median survival was 84 months. The worst category was represented by pts with low platelet count at diagnosis, who had a projected median survival of 38 months. This information might be useful in planning clinical trials with new drugs for Waldenstrom's macroglobulinemia.

P 59 THE INTERNATIONAL PROGNOSTIC INDEX (IPI) FOR LARGE-CELL LYMPHOMA IS ALSO USEFUL WHEN APPLIED TO PATIENTS WITH LOW-GRADE LYMPHOMA

A. López-Guillermo, E. Montserrat, F. Bosch, M. J. Terol, L. Escoda, J.C. Reverter, J. Ordi, J. Bladé, E. Carreras, F. Cervantes, P. Marín, B. Nomdedeu, M. Rovira, J. Sierra, A. Urbano-Ispizua, C. Rozman. Postgraduate School of Hematology "Farreras Valentí". Hospital Clínic. University of Barcelona, Barcelona, Spain.

In the analysis of prognostic factors in a series of 143 patients with low-grade lymphoma from our institution variables associated with a shorter survival were: age, performance status (PS), B symptoms, stage, number of lymph-node sites involved, extranodal involvement, bone marrow infiltration, ESR, WBC count, leukemic expression, and serum LDH. Of note, some of these variables (namely, age, PS, stage, number of extranodal sites, and serum LDH) are those employed to build up the IPI for large-cell lymphomas. Therefore, in order to determine whether the IPI is also useful in low-grade lymphomas, we applied this index to our series. Main results are shown in the table:

Risk Group	Patients (%) (n=125)	CR (%)	10 yrs Survival (%)
LOW	36.0	60	73.6
LOW/INTERMEDIATE	32.0	35	45.2
HIGH/INTERMEDIATE	20.8	23	53.5
HIGH	11.2	21	0

The prognostic value of IPI was further investigated by including it in a multivariate analysis along with the other variables previously identified. In such analysis it was found that IPI ($p<0.001$) and sex (male, worse) ($p=0.038$) were independent prognostic variables for survival. When response to treatment was included in the model, IPI retained its significance ($p<0.001$). Furthermore, IPI was the only parameter related to survival ($p=0.051$) in patients having achieved a CR.
In conclusion, in this study IPI has been found to be an important prognostic tool in low-grade lymphomas. If confirmed in other series, IPI could be used to predict prognosis not only in large-cell but also in low-grade lymphomas.

P 60 PROGNOSTIC FACTORS IN PATIENTS WITH NON HIV-RELATED PRIMARY CEREBRAL LYMPHOMA.

J.Y. Blay[1], B. Coiffier[2], C. Haioun[4], H. Tilly[5], C. Gisselbrecht[3], C. Carrie[1], Lasset C.[1], T. Philip[1], P. Biron [1]. 1-Département de chimiothérapie massive. Centre Léon Bérard,Lyon. 2-Centre Hospitalier Lyon-Sud,Pierre-Bénite. 3-Hôpital St Louis,Paris. 4-CH Henri Mondor,Créteil. 5-Centre H. Becquerel,Rouen. France

We analysed the survival of 81 patients (27F, 54M, median age: 57yrs) treated for a non HIV related primary cerebral non Hodgkin's lymphoma (PCL) in the Centre Léon Bérard (n=59) or in other institutions (n=32). 44 of these 81 patients were treated according in the LNH87 protocol. The median overall survival (MS) was 19 months with a median follow-up of 40 months. No significant differences in terms of age, histological subtypes and overall survival were observed between patients treated in or outside the CLB. The objectives were 1) to test our previously reported prognostic model on a larger serie of patients. 2) to evaluate the impact of the different chemotherapy regimens in the 3 risk groups. Among the 81 patients, three significant prognostic factors were identified in univariate analysis: CSF protein level before chemotherapy (<0.6g/l vs higher), age (<60 vs older), PS (ECOG scale: <3 vs higher), serum LDH and β2microglobulin, tumor size, histological subtype had no significant prognostic value. In a multivariate analysis, CSF protein, PS, and age were found to have an independent prognostic value. Using the regression coefficient of these three parameters, we were able to distinguish 3 risk groups with a discriminant survival (Risk group 1 (38% of patients, MS: 57 months), none or one of the 2 latter factors AND CSF Pr<0.6 g/l; Risk group 2 (26% of patients, MS: 16 months), both PS>2 and age>60 or CSF Pr>0.6 g/l alone, Risk group3 (36% of patients, MS: 5 months), all other patients.
Two therapeutic parameters were found to influence survival among the 81 patients. First, patients treated with third generation chemotherapy regimens (ACVBP in the LNH87 group 2 or 3, or COPADEM in the LMB) had a significantly longer overall survival (MS: 21 vs 13 months, p=0.04) compared to those treated with other chemotherapy regimens or no chemotherapy (the two latter subgroups were not significantly different). In second, regimens including 2 or more administrations of >1.5 g/m² of methotrexate were associated with a better survival than other protocols (MS: 27 vs 5 months, p=0.0001).
We then analysed these results acording to the above defined risk groups. Third generations regimens were associated with an improved survival in patients in group 1 but not in risk group 2 or 3. In contrast, treatment with HDMTX was associated with a better survival in all prognostic subgroups. These results confirm the validity of our prognostic model in a larger serie and indicate that intensive chemotherapy regimens may have a different impact in the 3 prognostic subgroups of primary cerebral lymphoma.

P 61 A NEW PROGNOSTIC INDEX INCLUDING S-CA125 AND LDH LEVELS IN PATIENTS WITH NON HODGKIN'S LYMPHOMAS.

L. Benboubker, C. Valat, C. Linassier, M. Delain, C. Petitdidier, F. Fetissof, JP. Lamagnere, Ph. Colombat
CHRU Bretoneau, 37044 Tours, France.

CA125 serum level (s-CA125) was measured by radio immunoassay (CIS international®) at diagnosis in 45 patients with non Hodgkin's lymphoma (NHL). They were 25 men and 20 women with a mean age of 58.2 years (range 24 - 80 years); Sixteen patients had a low grade histology whereas 29 had a high grade subtype. Ann Arbor stage was: IV in 23 patients (7 of which had more than one extra nodal site), III in 5 cases, II in 6 cases, and I in 11 cases. B-Symptoms were present at diagnosis in 5 patients and a bulky tumoral mass was found in 11 patients. All patients were homogeneously treated according to the Paris Ouest France protocols.
S-CA125 was abnormal (> 30 UI/ml) in 16 patients (35.6 %). S-CA125 level was closely related to the stage of the disease. In stage I-II, only 8 patients (11 %) had elevated s-CA125 level (mean value 21.7 ± 15.6 UI/ ml) whereas 14 patients (50 %) had an abnormal dosage in stage III-IV patients (mean value 259 ± 512 UI/ml) (p = 0.0037). LDH level was also related to the stage of disease. In stage I-II, only 6 % of patients had elevated level (mean value was 240.6 ± 65.2 UI/l) whereas 60 % had an abnormal dosage in stage III-IV patients (mean value 522.4 ± 359.6 UI/l) (p = 0.0014).
None of patients with stage I-II had both elevated s-CA125 and LDH levels increase, opposite to 40 % of patients with stage III-IV disease (p = 0.0005). We found no difference in s-CA125 levels between patients with either high grade or low grade NHL (p= 0.9).
All patients were assessable for response to induction therapy. Table 1 reports the early response after 3-4 courses of induction chemotherapy according to the LDH and CA125 levels at diagnosis. Complete remission (CR) rate was found significantly higher in patients with normal s-CA125 or LDH levels than in other patients. When taking both markers together, patients with normal LDH and s-CA125 levels responded better to therapy than patients with normal s-CA125 and elevated LDH levels and far more better than patients with both elevated markers. We conclude that the combination of s-CA125 and LDH is a good prognostic index in patients with NHL.

Table 1:

	s-CA125		LDH		s-CA125 + LDH		
UI/L	<30	>30	<300	>300	NN	NA	AA
#CR(%)	25 (86.2%)	6 (37.5 %)	22 (84.6 %)	6 (37.5 %)	20 (95.2 %)	8 (61.5 %)	3 (27.3 %)
#PR(%)	1 (3.4 %)	4 (25 %)	2 (7.7 %)	3 (18.7 %)	1 (4.8 %)	1 (7.7 %)	3 (27.3 %)
#Failure(%)	3 (10.4 %)	6 (37.5 %)	2 (7.7 %)	7 (43.8 %)	0 (0 %)	4 (30.8 %)	5 (45.4 %)
$\chi 2$	11.66		9.26		16.02		
p-value	0.0012		0.0098		0.0003		

CR : complete remission ; PR : partial remission ; N : normal ; A : Abnormal.

P 62 EVOLVING PROGNOSTIC FACTORS IN 411 CONSECUTIVE PATIENTS WITH AGGRESSIVE NON HODGKIN'S LYMPHOMA.

G. Palmieri*, M. Annunziata*, G. Abate#, M. De Marco*, S. Tafuto#, P. Fimiani#, A. Rea*, A. Morabito*, V. Ferrara*, A. Gravina*, G. Esposito*, P. Tagliaferri*, M. Salvatore*, M. Montella*, A.R. Bianco*.
Division of Oncology, Medical School II, University of Naples*, Division of Ematology# and Division of Epidemiology, National Cancer Institute* of Naples.

We have reviewed the pretreatment characteristics of 411 consecutive patients (pts) with aggressive Non Hodgkin's Lymphoma who were observed at our Institutions from 1977 to 1992. The patients population was divided into four groups according to time of diagnosis: group I pts diagnosed between 1977-1981 (no. 67); group II pts diagnosed between 1982-1985 (no. 104); group III pts diagnosed between 1986-1989 (no. 135); group IV pts diagnosed between 1990-1992 (no. 105). All pts were treated with ongoing chemotherapy programs. We evaluated the following pretreatment characteristics, which are thought to represent the most significant prognostic factors in aggressive Non Hodgkin's Lymphoma: age, stage, B symptoms, bulky disease, LDH levels, Hb levels, bone marrow involvement, number of extranodal sites. Results of our analysis are shown below:

	Group I	Group II	Group III	Group IV	
Age <40	6 (9%)	16 (15%)	33 (25%)	16 (15%)	
" 41-60	34 (51%)	52 (50%)	50 (37%)	52 (49%)	
" >60	27 (40%)	36 (35%)	52 (38%)	37 (36%)	
Stage I-II	25 (37%)	47 (45%)	42 (31%)	41 (39%)	
" III	19 (28%)	17 (16%)	31 (23%)	19 (18%)	
" IV	23 (35%)	40 (39%)	62 (46%)	45 (43%)	
B Symptoms	17 (25%)	27 (26%)	41 (30%)	33 (31%)	p<0.001
Bulky dis.	15 (22%)	42 (40%)	76 (56%)	64 (61%)	p<0.001
LDH >240	13 (20%)	42 (40%)	85 (63%)	62 (60%)	p<0.0001
Hb <10	18 (27%)	17 (16%)	34 (26%)	16 (15%)	
Marrow inv.	9 (13%)	23 (22%)	27 (20%)	26 (24%)	
0 Extr.sit.	32 (47%)	48 (46%)	44 (32%)	19 (18%)	
1 Extr.sit.	25 (37%)	39 (38%)	58 (43%)	54 (52%)	
>1 Extr.sit.	10 (16%)	17 (16%)	33 (25%)	32 (30%)	

These results reconfirmed our previous data indicating a changing pattern in the presentation of aggressive lymphomas, with an increasing incidence of some unfavorable prognostic factors. Our observation may lead to a riconsideration of therapeutic programs of this group of diseases. Given the high morbidity and mortality associated with the more effective new regimens, precise risk characterisation is mandatory to allow the design of patient-tailored programmes with the use of less toxic regimens in patients with a lower risk of relapse and of more effective intensive regimens in patients with a worse prognosis.

P 63 DISSEMINATED GROWTH OF HODGKIN DERIVED CELL LINES IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE

Ursula Kapp, Andreas Düx, Elizabeth Schell-Frederick, Michael Hummel, Susanne Mücke, Jörn Bullerdiek, Claudia Gottstein, Andreas Engert, Volker Diehl and Jürgen Wolf

Local tumor growth has been reported after subcutaneous and intraperitoneal injection of Hodgkin derived cell lines into different immunodeficient mouse strains. Since new immunotherapeutic strategies will be employed in patients with disseminated disease, an animal model with disseminated growth of tumor cells would be useful for preclinical testing. Therefore, the Hodgkin derived cell lines L540, L540cy, L428 and KM-H2 were injected intravenously into SCID mice. In contrast to L428 and KM-H2, widespread neoplasia occurred after a period of 4-6 weeks following injection of L540 and the subline L540cy. The lymph nodes were found to be the preferred site of tumor growth. The CD30 surface antigen on Hodgkin cells and the karyotype of the cells were preserved in the animal host. Thus, the SCID mouse model mimics to a large extent the dissemination pattern of Hodgkin's disease in man and may provide a useful tool for evaluation of the efficacy of conventional and newly developed therapies.
To evaluate the role of adhesion molecule expression in the dissemination of Hodgkin-derived cell lines, CD44 and members of the immunoglobulin, integrin, selectin and Fc receptor families were quantified by flow cytometry. CD30 expression was also measured. Although CD44 expression has been correlated with dissemination in non-Hodgkin lymphoma, this was not the case in the Hodgkin SCID mouse model. CD44 was not expressed on the disseminating cell lines L540 and L540cy.

P 64 THE B7/BB1 ANTIGEN IS EXPRESSED BY REED-STERNBERG CELLS OF HODGKIN'S DISEASE: FURTHER EVIDENCE FOR AN ACCESSORY CELL FUNCTION OF REED-STERNBERG CELLS.

J. Delabie, P. Vandenberghe, J.L. Ceuppens, C. De Wolf-Peeters.
Departments of Pathology and Internal Medicine (Div. of Clinical Immunology), Catholic University of Leuven, B-3000 Leuven, Belgium

The B7/BB1 molecule recently received much attention, because it has been found to be the natural ligand for CD28, expressed by T cells. CD28 provides upon binding of B7/BB1 a signal that synergizes with the T cell antigen receptor to induce T-cells to proliferate and secrete cytokines and that prevents anergy induction of T cells.
Since Reed-Sternberg cells are known to be strong stimulators in mixed lymphocyte reactions, we evaluated the expression of B7/BB1 in lymph nodes affected by Hodgkin's disease. In addition, non-Hodgkin's lymphomas, including T cell rich B cell lymphomas, were evaluated for B7/BB1 expression. B7/BB1 was found to be strongly expressed by the Reed-Sternberg cells in all cases of Hodgkin's disease studied, irrespective of the subtype of Hodgkin's disease. In contrast, B7/BB1 is not expressed by the neoplastic cells in the majority of non-Hodgkin's lymphomas including T-cell rich B-cell lymphoma. Evidence for a functional role of B7/BB1 on Reed-Sternberg cells was obtained by our findings that the primary allogeneic mixed lymphocyte reaction using the B7/BB1 expressing Hodgkin's disease cell lines L428 and KM-H2 as stimulators, could be partially blocked by adding anti-B7/BB1 antibody.
Our data provide further evidence for an accessory cell function of Reed-Sternberg cells and strongly indicates that the accumulation of reactive T cells observed in Hodgkin's disease involves different mechanisms than those acting in non-Hodgkin's lymphomas.

P 65 PROLIFERATION OF STERNBERG-REED CELLS IN HODGKIN'S DISEASE.

R. K. Gupta, J. G. Bodmer and T. A. Lister. ICRF Department of Medical Oncology, St Bartholomew's Hospital, London & Tissue Antigen Laboratory, Imperial Cancer Research Fund. London, England. (U.K.).

The proliferative activity and the origin of multinucleated Sternberg-Reed cells in Hodgkin's disease have been studied using cell cultures, thymidine incorporation and immunohistological techniques. The presence of both proliferating cell nuclear antigen (PCNA) and the Ki-67 antigen associated with cell proliferation have been reported in Hodgkin's disease. However, PC10 (an antibody to PCNA) may not reflect the true proliferating fraction as results may vary with antibody concentration and the use of Ki-67 has been limited to frozen material.

$p34^{cdc2}$ is the protein product of the cell cycle control cdc2 gene. The $p34^{cdc2}$ kinase functions at cell cycle control points and is necessary for mitosis. It also operates in G1 and is involved in the commitment of cells into the proliferative cycle. Using a monoclonal antibody against the protein p34 (courtesy T.Hunt), cases of different histological subtypes of Hodgkin's disease have been studied along with normal tonsil and follicular lymphoma as controls. As expected, positive immunostaining in the controls with anti-p34 was reciprocal to that found using an anti-bcl-2 antibody.

In the ten cases of Hodgkin's disease, positive p34 staining was seen in the majority of Sternberg-Reed cells and mononuclear variants (>90%), along with a proportion of small lymphocytes, mainly T-cells. Staining was predominantly cytoplasmic and occasionally additional nuclear signals were seen.

In addition, double-stained sections with the anti-p34 antibody and CM-1 for p53 were examined. In these cases, positive signal for both proteins were seen in the same cells. Whether the presence of p34 in Sternberg-Reed cells reflects mitosis or nuclear division without cell division (endomitosis) and hence explain the multinucleated appearance of these cells, remains to be determined.

P 66 CLINICAL FEATURES PREDICTING BONE-MARROW INVOLVEMENT IN HODGKIN'S DISEASE : ATTEMPT TO AVOID UNNECESSARY INVESTIGATIONS IN FAVOURABLE PRESENTATIONS.

H. Eghbali, F. Bonichon, C. Bonnel, P. Soubeyran, I. de Mascarel and B. Hœrni. Fondation Bergonié, 180, rue de Saint-Genès 33076 Bordeaux Cedex (France)

Bone-marrow biopsy (BMB) is widely performed in untreated Hodgkin's Disease (HD) staging in order to choose the most appropriate treatment according to clinical classification. It is commonly admitted that bone-marrow is involved in 5 to 15 % of cases in HD, but it is also obvious that bone-marrow involvement (BMI) is in relation with clinical extension and disease aggressiveness. In order to assess this relation, we reviewed clinical and routine biological features of 261 unselected newly diagnosed HD before randomized trials. After clinical examination there were 157 patients (pts) with no systemic symptoms according to Ann Arbor classification criteria (A = group A) and 104 pts with fever and/or night sweats and/or weight loss more than 10 % (B = group B) respectively 61 and 39 % of pts. Bone-marrow was involved in one case of group A (0.6 %) and in 14 cases of group B (13.5 %). In the second step, the analysis of radiological features (chest X-ray, CT scan) before BMB showed 3 cases of liver and lung involvement in group A and 16 cases in group B. The only case of BMI in group A had liver involvement and generalized lymphadenopathy. In group B, regardless to BMB, there were 9 cases of liver and/or lung involvement. After BMB all of these 9 pts had BMI and 5 had BMI without other visceral involvement. Statistical analyses (CHI2) conclude that the most predictive factor is : 1] B-symptoms (p value < 0.0001) ; 2] liver/lung involvement (p value < 0.0001) and more than 4 involved lymph node areas (p value < 0.0001). There is also obviously a correlation with positive lymphangiogram, clinical spleen enlargement and leukopenia or thrombopenia, but we didn't find correlation with age, histological type (2 vs 3) and erythrocyte sedimentation rate. These results suggest that BMB should be reserved for all cases of B-symptoms but it is unnecessary and useless in clinical stages I/IIA with less than 4 lymph node areas. In this case there will be an additional benefit for patient's quality of life and health financial costs.

P 67 INTERLEUKIN-8, S-TNF RECEPTOR AND P53 PROTEIN LEVELS ARE ELEVATED IN SERA OF HODGKIN'S DISEASE PATIENTS.

L. Trümper, G. Dahl, A. Gause, W. Jung, V. Diehl, M. Pfreundschuh. Department of Internal Medicine I, University of Saarland, 6650 Homburg/Saar, and 1st Medical Clinic, University of Cologne, Germany

Hodgkin's disease patients frequently exhibit severe systemic symptoms even with a low tumour load. These symptoms are thought to be mediated by cytokines released by inflammatory bystander cells or the tumour cells themselves. In addition, surface molecules shed into the serum by the tumour cells may serve as tumour markers and may have prognostic significance as has been shown for the soluble form of the CD 30 molecule. We examined circulating levels of Interleukin-8 (IL-8), Interleukin 7 (IL-7), the soluble form of the p60 TNF receptor (sTNF-R) and circulating levels of the p53 growth suppressor protein in the sera of 40 (80 for IL-8) consecutive patients with Hodgkin's disease before therapy.

IL-8 levels were elevated in 20/80 sera; the mean serum level was 1190 pg/ml (range: 82-9648 pg/ml; normal controls: < 30 pg/ml). Elevated levels corresponded to advanced stages (III and IV acc. to Rye), the presence of B-symptoms (16/20 patients) and elevated white blood cell count (11/20 patients) when compared with the remaining 60 patients. Since IL-8 is stimulated by IL-1 and TNF and activates neutrophils, IL-8 in Hodgkin's sera may be responsible for the activation of granulopoiesis frequently seen in these patients. In contrast, serum levels for Il-7, a B-cell stimulatory cytokine, were not significantly elevated in the sera of 7/40 patients only. sTNF-R levels were elevated in 12/34 sera above 500 ng/ml, without correlation to stage or sCD 30 levels (elevated in 5/34 sera). Therefore, this parameter does not seem to have prognostic significance in Hodgkin's disease. Serum levels of p53, as assessed by a sandwich ELISA employing two different antibodies to different p53 protein conformations, were elevated in 7/34 sera. The presence of antibodies to p53 that may have prognostic significance as in other tumours is presently examined by immunofluorescence.

P 68 CLINICAL AND BIOLOGICAL RELATIONSHIPS WITH PATHOLOGY IN HODGKIN'S DISEASE : THE EORTC LYMPHOMA COOPERATIVE GROUP AND GROUPE PIERRE-ET-MARIE-CURIE EXPERIENCE.

A-M. Mandard, J. Bosq, K.A. MacLennan, J. Diebold, M.H. Bennett (Ψ), H. Beerman, Ph.M. Kluin, C. de Wolf-Peeters, A. Carbone, P. van Heerde, C. Duval, M. Trojani, L.W. Vrints, J. Jancar, E. van Marck, E.M. Noordijk, P. Carde, J.M.M. Raemaekers, J.M.V. Burgers, J. Marnay, N. Dupouy, A. Pinna and M. Henry-Amar. Service d'Anatomie Pathologique, Centre Régional François Baclesse, 14021 Caen cedex, France.

From November 1988 to December 1992, 967 clinical stage (CS) I-IV Hodgkin's disease (HD) adult pts from 48 centres in 8 european countries were prospectively enrolled in two ongoing phase III trials (EORTC protocols # 20881 and 20884). Usual staging procedures were performed including lymphangiography, but no laparotomy. Histologic material was prospectively reviewed by a panel of pathologists who assessed the diagnosis of HD. The Rye classification was used and, among the nodular sclerosing (NS) subtype, cases were classified according to the BNLI grades I (G1) or II (G2). Complete remission (CR) was assessed after initial treatment in supradiaphragmatic CS I-II pts, after 6 courses of chemotherapy in CS III-IV pts. Relationships were assessed using the Fisher exact test, one-way or two-way analysis of variance, as appropriate. Factors predicting for CR were assessed through a logistic regression model which included age, gender, stage, systemic symptoms, number of nodal and extra nodal localizations, mediastinal bulk, and biological parameters [erythrocyte sedimentation rate (ESR), haemoglobin (Hb), leucocytes, neutrophils, platelets, erythrocyte corpuscular volume (ECV) and γGT] as dependent variables.

Overall, there were 23% CS I, 50% CS II, 16% CS III and 11% CS IV pts. The sex ratio was 1.0 and 1.7 in CS I-II and CS III-IV pts, respectively ($p<0.001$). Mean age at diagnosis was 33.6 and 36.4 years ($p<0.01$), B symptoms were present in 23% and 59% ($p<0.001$) of the pts, respectively.

After review (588 CS I-II and 179 CS III-IV cases) by the histology panel, 94% of the cases were considered true HD, 2% were doubtful, 2% were unconclusive specimens, and 2% were classified as non-Hodgkin's lymphomas. Of the 722 true HD cases, 3% were lymphocytic predominant (LP), 79% were NS, and 18% were mixed cellularity (MC) subtype. Among the 573 NS cases, 59% were G1, 36% were G2, whereas 5% were borderline cases.

NS was associated with female gender ($p<0.001$) and CS II-IV ($p<0.001$). B symptoms were more often present in NS and MC than in LP ($p<0.01$) subtype. Among NS cases, G2 correlated with stage ($p<0.01$). NS and MC correlated with low Hb and high ESR ($p<0.001$). NS also correlated with high leucocytes, high neutrophils and high platelets ($p<0.001$), and low ECV ($p<0.01$). Among NS subtype, G2 correlated with low Hb and high ESR ($p<0.01$), high platelets ($p<0.001$), and low ECV ($p<0.05$). An absence of CR at the end of treatment was associated with low Hb ($p<0.01$) and low ECR ($p<0.01$) as well as with NS ($p=0.064$) subtype. The same biologic factors, but not G2, significantly ($p<0.05$) correlated with an absence of CR when the analysis was restricted to NS cases.

P 69 BETA-2-MICROBLOBULIN (β2-MG) : A GOOD PROGNOSTIC FACTOR FOR RESPONSE AND SURVIVAL IN YOUNG ADULTS WITH HODGKIN'S DISEASE (HD).

J. Fleury, M. Legros, J. Tortochaux, H. Curé, P. Travade, Y.J. Bignon, G. Gaillard, E. Bélembaogo, B. Chouli, F. Tavernier, F. Kwiatowski, I. van Praagh, Ph. Chollet, R. Plagne. Centre Jean Perrin, 63011 Clermont-Ferrand Cedex 1,

β2-MG appears a good indicator of responsiveness and survival in non Hodgkin's lymphomas (NHL) (Legros M *et al*, Proc. ASCO, 1987). We studied level from 84 previously untreated patients (55 males, 29 females) less than 50 years affected by HD. Serum β2-MG level was measured by radioimmuno-assay (normal range : 1.6 to 2.4 mg/l). Thirty-seven were stage I-II and 47 stage III-IV according to Ann Arbor classification. Fourty-three had systemic symptoms, 25 bulky disease, 54 biological stage b. Nodular sclerosing (n = 33) and mixed cellularity (n = 32) were predominant in Lukes-Rye classification. Patients were treated with chemotherapy alone or combined with radiotherapy. Chemotherapy regimens consisted of MOPP alone or alternating MOPP/ABVD.

The response and survival rates (Kaplan-Meier's method) are summarized as below :

	CR after 3 courses	CR at the completion of treatment	Relapse after CR	Primary Refractory	Overall surv. 5 yr	DFS 5 yr
β2-MG ≤ 2.4 (n = 65)	37/60 (61 %)	58/63 (89 %)	4 (7 %)	5 (7.5 %)	89,1 %	85,5 %
β2-MG > 2.4 (n = 19)	4/18 (22 %)	12/18 (66 %)	4 (33 %)	4 (21 %)	65.2 %	55.3 %
p (chi-2)	< 0.02	< 0.10	< 0.10	NS	< 0.03	< 0.01

For all patients the median follow-up was 65 months (5-179).

A multivariate analysis (Cox model) was performed and included : age, sex, presence or absence of systemic symptoms, clinical stage (I-II, III-IV), tumor burden, biological stage, histology and initial seric β2-MG rate (≤ 2.4 mg/l or > 2.4 mg/l).

	Early response (after 3 courses)	Final response	Overall survival	Disease-free survival
Significant variables	β2-MG Sex	Systemic symptoms Sex	Clinical stage	β2-MG Clinical stage

The prognostic value of β2-MG was also found for stage I-II and stage III-IV. Moreover we noted a significant correlation between β2-MG rate and systemic symptoms ($p < 0.01$), bulky tumor ($p < 0.01$) and clinical stage ($p < 0.02$). No correlation was found with other biological parameters.

In conclusion, initial seric β2-MG appears in this study a good indicator of chemosensibility and survival for young adults with HD.

P 70 VBM (VINBLASTINE, BLEOMYCIN, METHOTREXATE) CHEMOTHERAPY WITH INVOLVED FIELD RADIOTHERAPY IN THE MANAGEMENT OF "EARLY" HODGKIN'S DISEASE.

N. P. Bates, M. V. Williams, E. M. Bessell, G. Vaughan Hudson & B. Vaughan Hudson for the British National Lymphoma Investigation (BNLI), Middlesex Hospital, Mortimer Street, London.

The VBM regimen (Vinblastine, Bleomycin, Methotrexate) was reported by Horning et al. (1988, J. Clin Oncol. 6;1822-1831) to prevent relapse after involved field irradiation in laparotomy staged patients with PS IA and IIA Hodgkin's disease.

To assess the VBM in unselected clinically staged patients the BNLI initiated a prospective, multicentre pilot study. Two cycles of VBM were given prior to involved field irradiation (40 Gy in 20 fractions). Four more cycles of chemotherapy were then given to complete the planned treatment.

30 eligible patients were enrolled in a year. Response to chemotherapy was assessed at 6 weeks (after two cycles of VBM). 26 patients had assessable disease and all showed an objective response to therapy: 9 showed a clinical complete response (not confirmed by CT scans) and 17 showed an objective partial response. At the completion of all therapy 28/30 patients have achieved complete remission and two have stable residual masses. Follow-up ranges from 18-30 months off therapy and one patient has relapsed at 19 months.

Cough, dyspnoea and abnormal pulmonary function tests occurred in 50% of subjects and led to the discontinuation of Bleomycin therapy. This toxicity interfered with normal activity, but was reversible. 22/30 patients received mediastinal irradiation. In those whose mediastinum was irradiated lung toxicity occurred in 13/22 (60%); without mediastinal irradiation lung toxicity was seen in 1/8 (12%). These differences were not significant ($p=0.06$). Three episodes of neutropenic sepsis occurred, one of which was fatal.

In our hands the VBM regimen with intercalated irradiation is effective but produced unacceptable pulmonary toxicity.

P 71 HODGKIN'S DISEASE (HD) WITH A MEDIASTINAL MASS GREATER THAN 10 CM: RESULTS OF FOUR DIFFERENT TREATMENT APPROACHES.

A. Preti, F. Hagemeister, F. Swan, M. Rodriguez, P. McLaughlin, A. Younes, P.K. Allen, A. Sarris and F. Cabanillas. U.T. M.D. Anderson Cancer Center, Houston, Texas 77030.

Treatment of patients (pt) with HD and large mediastinal masses (MM) traditionally includes extensive chemotherapy (CT) with or without radiation therapy (XT) regardless of stage. From 1970 to 1990, 137 newly diagnosed pt with MM greater than 10 cm received therapy at our institution. We excluded 19 patients from review because of protocol violation or incomplete records. Characteristics of the 118 evaluable pt included: female-52%, Nodular Sclerosis subtype-92%, median age-26 years (range 14 to 64); stages I-III 78%; B symptoms-56%; and hilar involvement-33%. Pt with stages I-III received one of four treatment regimens: (1) 6 to 8 cycles of MOPP or similar CT plus XT, (2) 2 cycles of MOPP or 3 of ABVD plus XT, (3) 6 of CVPP/ABDIC (C/A) (cyclophosphamide, vincristine, procarbazine, prednisone/ doxorubicin, bleomycin, dacarbazine, lomustine) plus XT, or (4) 3 of NOVP (mitoxantrone, vincristine, vinblastine, procarbazine) plus XT. XT doses included 30 Gy to areas of nodal involvement prior to therapy. By protocol design, no patient with stage IV disease received NOVP plus XT; the incidence of B symptoms was also different for those receiving 6 MOPP (53%), 2 MOPP (48%), C/A (25%), and NOVP (30%). Complete remission (CR), freedom from progression (FFP), and freedom from tumor mortality (FTM) rates are shown:

Treatment	Stage	No of Pts	CR %	3 yr FFP %	3-4 yr FTM %
6 MOPP plus XT	II-III	8	100	88	100
	IV	5	60	20	80
6 C/A plus XT	II-III	28	87	82	84
	IV	19	80	59	94
2 MOPP plus XT	I-III	32	85	66	84
3 NOVP plus XT	II-III	26	96	88	100

FFP results for those receiving NOVP with and without B symptoms were 75% and 94%, respectively. None of the above comparisons are statistically different. From this analysis, we propose that it is unnecessary to administer 6 cycles of CT to patients with MM greater than 10 cm with early staged HD, since it appears that less CT provides FFP and FTM results that are as good as those achieved with 6 cycles in combined modality programs.

P 72 ANALYSIS OF TREATMENT OUTCOME AFTER EBVP CHEMOTHERAPY AND INVOLVED-FIELD IRRADIATION ACCORDING TO PROGNOSTIC FACTORS IN HODGKIN'S DISEASE STAGES I AND II.

H. Eghbali, P. Richaud, P. Soubeyran, F. Bonichon and B. Hœrni. Fondation Bergonié, 180, rue de Saint-Genès 33076 Bordeaux Cedex (France)

The main problem of Hodgkin's Disease (HD) treatment in past years and especially now, is the necessity of cure and avoidance of early and late complications such as aplasia, sterility or acute leukemia. In this purpose, ABVD-derived EBVP (epirubicin, bleomycine, vinblastin, prednisone) is used in order to reduce side-effect vomiting of dacarbazine and in the same time late toxicity of alkylating drugs (myelodysplastic syndrome, leukemia, sterility) in MOPP or CVPP. The early results of this regimen were as good as ABVD and MOPP, all three used in a sandwich-framed protocol including induction chemotherapy (CT), involved-field radiotherapy (IF) and consolidation CT. Recently in the EORTC phase III trial H7, it was suggested that EBVP may be inadequate for unfavourable cases (ASCO Proceedings 1993). In order to check this assumption, we carried out a retrospective analysis of all patients (pts) with clinical stage I, II treated by EBVP + IF radiotherapy. One hundred and ten pts of 14 to 75 years (median 33) were reviewed and divided into two groups favourable (F) and unfavourable (U) according to EORTC prognostic factors which are : 1] age ± 50 years ; 2] number of involved lymph node ≤ 3 vs > 3, systemic symptoms B vs A ; 3] ESR < 50 mm vs ≥ 50 if A or < 30 vs ≥ 30 if B ; 4] bulky mediastinum < 0.35 vs > 0.35, histology 1-2 vs 3. Thus, 52 pts of U group and 58 pts of F group were compared. All were clinically staged without laparotomy but with CT scan, lymphangiogram, bone-marrow biopsy and whole biological work-up. The treatment consisted on 3 courses of EBVP before and after IF irradiation. The total CT dosage was the same in two groups with an average of 337 mg/m^2 for epirubicin, 66 mg/m^2 for bleomycine and 40 mg/m^2 for vinblastin. All of them were irradiated only on IF areas. Complete remission was achieved in 53 cases (91 %) for F and 50 cases (96 %) for U group after EBVP or after radiotherapy (58 and 51 pts). One patient failed in group F, 8 pts relapsed in F and 9 in U group (p value 0.4) and 8 died of HD (F: 2 ; U : 6 – p value 0.058). According to Kaplan-Meier method and Log rank test and with a median follow-up of 7 years, disease-free survival is respectively 90% for F and 84 % for U. These results with a **long follow-up** suggest : 1] despite a priori worse prognosis, the U group has the same relapse-free survival ; 2] relapses are sooner in U group, and late in F group. Thus early relapses are not significant if compared ; 3] more than 80 % of all pts are cured by a non leukemogenic, non myelotoxic and well tolerated regimen. They shouldn't be forgotten ; 4] in all HD trials a long follow-up is absolutely necessary before concluding.