ABNORMAL ABDOMINAL CT PREDICTS PROGRESSION AND SURVIVAL IN RAI STAGE 0 CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Purpose: To analyze the prognostic value of the abdominal CT in patients [pts] diagnosed with CLL in early clinical stage.

Patients and methods: Abdominal CT was performed in 90 pts (55M/35F; median age, 68 years) with CLL in Rai's stage 0. Five abdominal lymphoid areas were considered: diaphragmatic, celiac, mesenteric, retroperitoneal, and iliac. The image was considered as abnormal when lymph nodes larger than 1.5 cm in diameter or an increased number of smaller lymphatic nodes in one area were observed. Progression was defined as the change to a more advanced clinical stage or treatment requirement. Time to progression (TTP) was calculated from the time of the CT. Median follow-up was 43 months.

Results: An abnormal abdominal CT was found in 25 pts (28%) (retroperitoneal, 18%; iliac, 17%; celiac and mesenteric, 12%; diaphragmatic, 5%). All but three cases had lymph nodes of 2 cm or less. Among the main initial characteristics, abnormal CT imaging only correlated with bone marrow infiltration (normal vs. abnormal CT: 42% vs. 61% lymphoid infiltration; p=0.001) and overall progression rate at 48 months was 30%. Pts with abdominal CT progressed more frequently and had a shorter TTP (median TTP 29 months) than those with normal CT (median TTP not reached); p=0.001. In a Cox model, only the lymphocyte doubling time <12 months (p=0.001; RR=9.6) and the abnormal CT (p=0.001; RR=7.5) were correlated with disease progression. Survival at 6 years was 84%. Pts with more than one abnormal lymphoid area involved (n=17 [19%]) had shorter survival (p=0.05) than those with one area or normal CT.

Conclusions: In this series, abdominal abdominal CT is a strong predictor of progression and survival in patients with Rai's stage 0 CLL. Therefore, this examination should be included in the workup of patients with CLL in early stage.

Pulsed emission tomography (PET) scanning can replace routine staging marrow biopsy in Hodgkin’s Disease.

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Introduction. Bone marrow biopsy is a standard part of staging newly diagnosed Hodgkin’s Disease, but marrow involvement is identified in only 5-14% of cases. We have previously demonstrated that FDG-PET is an effective way of identifying marrow disease in both HD and NHL when the PET scans are specifically examined for increased uptake in marrow bearing bones [Carr et al Blood 1998;91:3345-3349]. The present study extends and confirms the usefulness of routinely reported PET for identifying marrow involvement by HD.

Method and Results. We examined the case records of 30 consecutive patients with HD treated in our centre, who had pre-treatment staging PET scans. Nineteen had had unilateral iliac crest staging marrow biopsies. We compared the results of the routinely reported PET scans with the bone marrow biopsy reports of these 19 patients.

12 (63%) showed no marrow involvement by either technique. 7 patients had marrow considered abnormal on PET, of which 3 (16%) had focal areas of high FDG uptake, but normal iliac crest marrow histology. (In one of these a “hot spot” in the humerus was biopsied and HD confirmed). 4 (21%) had diffusely increased FDG uptake: in 2 of these the iliac crest biopsy showed HD (Nodular Sclerosis: Mixed Cellularity); in the other 2 the marrow showed reactive myeloid hyperplasia x 2 eosinophilia, without evidence of HD.

Conclusion. PET is already established as an accurate non-invasive method of staging nodal HD [Partridge et al Ann Oncol 2000, 11:1273-1279 ] . These data confirm that PET is also a sensitive method for identifying marrow involvement, in contrast to iliac crest marrow biopsy which is painful and may miss disease if the infiltration is patchy or the biopsy inadequate.

These data confirm our previous proposition that:
1) Patients with negative PET on bone marrow PET do not need confirmation by biopsy.
2) Patients with diffusely positive marrow PET on PET need a marrow biopsy to differentiate between disease and reactive myeloid hyperplasia.

Using this approach, 5 of these 13 patients would have been accurately identified as having stage IV (marrow) HD, but only 4 would have been subjected to marrow biopsy.

FDG-PET VS 111Ga SCINTIGRAPHY AS A PROGNOSTIC TEST DURING CHEMOTHERAPY FOR NON HODGKIN’S LYMPHOMA


Introduction: In NHL, an important challenge to diagnostic tests is their ability to stratify patients (pts) into prognostic subsets. Since either FDG using Positron Emission Tomography (PET) or Ga could be suited for this purpose, the present study aimed to investigate which technique might be preferable with respect to test performance and observer variation, if applied after only 2 cycles of CHOP.

Methods: In 27 intermediate-risk NHL pts, 111Ga (10mCi, 3h post injection planar SPECT) and 18FDG WB-PET (10mCi, 60min post injection, HR+ H) scans were read by 2 couples of experienced nuclear physicians, independently and blinded for follow-up (FU). Data are presented as positive/negative for tumour activity per patient, as defined in a consensus reading of each team of observers. Clinical FU was provided by clinicians, blinded for scan results.

Results: During ongoing FU of 11-34 months, 12 pts (44%) had recurrent disease, 1 patient died of lung cancer and 14 are still in remission. For PET and Ga, the observers disagreed in 3 and 9 pts respectively with respect to the final examination. In a patient based analysis, Ga and 18FDG-PET were discordant in 30% (table). The likelihood ratios of positive PET and Ga scintigraphy were 2.2 and 1.5, respectively (NS). False negative PET results occurred in pts classified as low-grade NHL (n=2), in CNS relapse (n=2) and in late relapse (n=12 months, n=1).

Conclusion: FDG-PET seems to be the more reliable predictor of outcome after chemotherapy.

Aim: As we had access to a dedicated PET scanner for several months, we evaluated the results of PET clinical examinations for patients with lymphomas and those by gallium scintigraphy and compared the two methods wherever this was possible.

Method: The haematologists were free to prescribe the examination that seemed most appropriate. Because of administrative procedures and the timetable for PET use, 4 weeks were needed to obtain an appointment and accordingly the indications were restricted to an evaluation of the response to treatment. The PET was conducted using a Siemens CTI ECAT scanner 1 hour after intravenous injection of 370 MBq of Fluorodeoxyglucose, without transmission scan, then using a GE Advance with transmission scan. The gallium scintigraphy was performed using a DST XLI (SMV) gamma-camera 48 or 72 hours after injection of 185 MBq of gallium citrate. A bone scintigraphy was sometimes performed before this scintigraphy.

Results: Forty-eight PET were conducted on 41 patients. Nineteen of these patients had received from 1 to 5 gallium scintigraphies. Comparisons between the two methods were possible for 15 examinations in 13 patients - 9 Hodgkin’s diseases and 4 non-Hodgkin’s lymphomas. A difference in favour of the FDG was obtained in 2 patients (a residual sub-clavicular mass in 1 case, a mediastinal in another, with normal gallium scintigraphies). There was agreement in 10 cases (25%) and in 6 patients, abnormal ones at the same site in 4 other cases for 3 patients). There was partial agreement in 3 cases (a 4th site detected with FDG not seen with gallium in 1 patient, more additional sites with FDG in the last patient studied 2 times). All the sites were more clearly visible and easier to locate with FDG. There were no false positive with gallium.

Conclusion: Only 40 % of the patients who were given a PET also received a gallium scintigraphy. Prescriptions for the PET therefore seemed to be more frequent than those of gallium scintigraphy. The results of FDG in the evaluation of the response to therapy were better than those of gallium, but in a situation where access to PET is limited, gallium could be used as a first resort and FDG could be used only when the gallium scintigraphy is negative.

COMPARISON OF FDG PET AND GALLIUM SCINTIGRAPHY IN LYMPHOMAS


Aim: As we had access to a dedicated PET scanner for several months, we evaluated the results of PET clinical examinations for patients with lymphomas and those by gallium scintigraphy and compared the two methods wherever this was possible.

Method: The haematologists were free to prescribe the examination that seemed most appropriate. Because of administrative procedures and the timetable for PET use, 4 weeks were needed to obtain an appointment and accordingly the indications were restricted to an evaluation of the response to treatment. The PET was conducted using a Siemens CTI ECAT scanner 1 hour after intravenous injection of 370 MBq of Fluorodeoxyglucose, without transmission scan, then using a GE Advance with transmission scan. The gallium scintigraphy was performed using a DST XLI (SMV) gamma-camera 48 or 72 hours after injection of 185 MBq of gallium citrate. A bone scintigraphy was sometimes performed before this scintigraphy.

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18-F-FDG PET in monitoring therapy response to RIT by 131-I Rituximab (Matthera\textsuperscript{a}) antibody

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Introduction: To monitor early treatment response by radioimmunotherapy RIT using 131-I-labeled anti-CD20 Matthera antibody FDG PET is regarded if initially positive as gold standard. This study is concerned with the pretherapeutical and post therapeutical differences of FDG uptake induced by anti-CD20 RIT.

Methods: 1-2 weeks prior RIT and 4-6 weeks after RIT 15 patients (intermediate or high-grade B-cell lymphoma) underwent 18-F-FDG PET (dedicated PET scanner Siemens ECAT EXACT 47). Pathological tracer accumulations were evaluated by means of SUV and consecutive morphological imaging (CT/MRI).

Results: 10/15 patients did show a substantial reduction of numbers of lesions and in addition a decrease of SUV. 4 patients did show only slight reduction in SUV (<25%)

Conclusions: 18-F-FDG PET is if initially positive a very promising tool for early detection of response. Its predictive value in terms of over all survival has to be evaluated in further trials.
MAGNETIC RESONANCE IMAGES IN THE ASSESSMENT OF RESIDUAL MASSES OF NODULAR SCLEROSIS SUBTYPE OF HODGKIN DISEASE.
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Within the last 3 years, 31 patients of nodular sclerosing subtype of HD were treated since diagnosis at Department of Haematology in Częstochowa (6 low risk cases defined as I/II clinical stage with no risk factors, 14 intermediate risk cases and 11 patients in clinical stage III/IV). Our therapy protocols, based on EBVP and EBMT, included chemotherapy (EBVP, ABVD or BEACOPP) followed by IFPT where applicable. High-dose therapy (BEAM) followed by autologous BMT was performed in 3 early relapses and 4 high risk cases. Further 12 transplanted cases were referred to our center: 6 of them in primary resistance (after failing 2-3 previous chemotherapy regimens), 6 in 2-4th relapse.

The standard follow-up regimen included 3-monthly chest CT scans. Magnetic resonance investigation (T1, T2 and PD weighted images) was performed in 17/20 patients with non-progressive residual masses (RM) visible on the chest CT scans 6 - 24 months after the end of the therapy. In 2 cases typical areas of increased T2 weighted intensity in a low intensity residual masses were found indicating recurrence. It made us change the treatment plan and start the transplant procedure, before the progression could be detected by other techniques (as it happened in one of them, despite therapy, 3 months later). In 8 patients different patterns of signal (lesions of slightly higher intensity or inhomogeneous masses) indicated the need for sequential NMR scanning. Further 7 cases, with low intensity T2 and PD2 weighted images were regarded NMR negative, corresponding to fibrosis.

In our current protocol we are investigating the pragmatic implications of repeated NMR scans, done before, during and after therapy. In our experience, NMR imaging seems to be an additional, valuable tool in evaluating non-progressive residual masses, crucial for taking therapeutic decisions.

IMAGING OF CUTANEOUS T-CELL LYMPHOMA (CTCL) USING 18-F-FDG-POSITRON EMISSION TOMOGRAPHY (PET)
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Introduction: Primary cutaneous lymphomas (CL) are the second most common group of extranodal non-Hodgkin's lymphomas, with T-cell lymphomas (CTCL) accounting for 60% to 65% of all CL. The most common form is mycosis fungoides (MF), representing 70% of all cases of CTCL. MF usually shows an indolent clinical course, with slow progression over years, from patches to more infiltrated plaques and eventually tumors. Staging and follow up of MF patients is mainly restricted to conventional imaging modalities, whereas for all other forms of primary non-Hodgkin's lymphomas, whole body positron emission tomography using fluorodeoxyglucose (18-FDG-PET) has been shown to be highly sensitive and specific for imaging and monitoring of lesions.

Patients and Methods: Two patients, one male patient with MF, stage IVb and one female patient with plaque stage MF (Ib) were selected for scanning with whole body 18-FDG-PET. Cutaneous lesions could be visualized, and correlated with the clinically visible areas of involvement.

Conclusions: Staging and monitoring of CTCL patients have generally been restricted to sonography, X-ray and computed tomography. Evaluation of 18-FDG-PET for the monitoring of CTCL has not been reported so far. Our observations suggest, that 18-FDG-PET might contribute to more exact staging and follow-up of MF and other forms of CL.
3. Biology/ Genetics

Serum Thymidine Kinase and Soluble Interleukin-2 Receptor Predict Recurrence of Malignant Lymphoma

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Before and after therapy, serum thymidine kinase (TK) and soluble interleukin-2 receptor (sIL-2R) were serially determined in 28 patients with malignant lymphoma (ML). In 15 patients achieving and maintaining complete remission (CR) for more than 2 years, serum TK and sIL-2R were unchanged or decreased gradually. In contrast, logarithmic linear increases of TK and sIL-2R were observed in 13 relapsed patients. The increments of the serum markers preceded more than 10 months before the relapse. A significant positive correlation between the slope of the line for TK and that for sIL-2R was noted. The doubling time for TK estimated from the slope also showed a positive correlation with that for sIL-2R. Taken together, serum TK and sIL-2R were shown to be quite sensitive and interrelated serum markers for the recurrence of ML. Slopes of logarithmic linear increase, which are proper and specific for the individual patients, are inversely correlated with the doubling time and reflect proliferation of ML.

Analysis of Bcl-10-Interacting proteins CARD-10, CARD-11, CARD-14 & MALT-1 in reactive and malignant lymphoid lesions.

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Characterisation of t(1;14) in MALT lymphoma led to the identification of Bcl-10 and subsequently a family of proteins that contain the caspase recruitment domain (CARD), that is required in interaction with Bcl-10. Bcl-10 has also been shown to interact with MALT-1, a protein involved in t(11;18) MALT lymphoma. We investigated the involvement of CARD-10, CARD-11, CARD-14 and MALT-1 in reactive and malignant lymphoid lesions using immuno-histochemistry and real-time-PCR analyses. Analyses of a series of reactive lymphoid lesions show that CARD-10, -11 and MALT-1 are highly expressed in germinal centre (GC) B-cells, whereas CARD-14 is expressed in dendritic cells, T cells and macrophages. This finding was further confirmed by Western blot analysis using cell lysates from isolated T-cells, B-cells and macrophages from healthy blood donors and sorted B-cell populations from human tonsils. Analyses of a series of cases of malignant lymphoma have shown that CARD-10, -11 and MALT-1 are all expressed in tumours with presumed GC-origin. In 11 of 11 follicular lymphomas and 8 of 8 diffuse large B-cell lymphomas. This finding suggests that CARD-10, CARD-11 and MALT-1 could be used as GC B-cell markers. In addition, in vitro experiments suggest that these proteins are involved in a specific signal transduction pathway leading to NF-κB activation which has been reported to be critical for survival of lymphoma cells.

Withdrawn

MUM1/IRF4 EXPRESSION AS AN UNFAVORABLE PROGNOSTIC FACTOR FOR B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)/SMALL LYMPHOCYTIC LYMPHOMA (SLL)

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Introduction: B-CLL/SLL shows heterogeneity in terms of MUM1 expression, which is a protooncogene product deregulated as a result of t(6;14)(p21;q32) in multiple myeloma. We pursued the significance of the MUM1 expression in association with status of somatic hypermutation (SH) of the immunoglobulin heavy chain variable region (IgVH), CD38 expression and various clinical information of Japanese B-CLL/SLL patients.

Methods: MUM1 expression was examined by immunohistochemical analysis in 4 B-CLL derived cell lines and by immunohistochemistry in formalin-fixed specimens from 29 B-CLL/SLL patients at initial diagnosis. MUM1 positivity in immunostaining analysis was defined when more than 20% of the tumor cells were stained in their nuclei, as previously described (Tsuboi, et al., Leukemia 14:449, 2000).

Result: 2 of the 4 B-CLL cell lines and 14 out of 29 patients' specimens expressed MUM1. Its expression was not associated with CD38 expression, IgVH status, or any other patients' clinical characteristics. Interestingly, however, the patients positive for MUM1 showed shorter overall survival time than those with negative (50% survival at SE: 22±16 months vs. 82±35 months, P = 0.008 by log-rank test). Multivariate analysis by Cox's proportional-hazards model showed that MUM1 expression was an independent unfavorable prognostic factor as well as the unmutated IgVH status. These findings suggest that MUM1 is a worthy prognostic factor for B-CLL/SLL.
ADHESION MOLECULES ON HUMAN MYELOMA (MM) CELLS MIGHT CLARIFY DIFFERENT CLINICAL OUTCOME.


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Introduction: Some of the characteristic features of natural history of multiple myeloma (MM) implies the existence of mechanisms that allow a specific narrow involvement of myeloma plasma cells (PC). Both the degree of marrow infiltration and the diffusion of the disease to peripheral blood are unfavorable prognostic factors in MM. Little is known about factors that regulate plasma cell compartmentalization, although several lines of evidence suggest that adhesion molecules play a role in this process.

Methods: In this study, using a panel of specific monoclonal antibodies, we investigated the expression of CD38, CD138 ( Syndecan-1), CD56 molecule (NCAM) and the c-kit proto-oncogene CD117 in 18 patients with MM (7 men and 11 women - age from 38 to 90 years) at diagnosis and in 3 myeloma cell lines: U2-66, RPMI-8226 and OPM-2. Among the 18 patients, 10 were Iga isotype, 5 were Iga isotype and 3 did not show any gammopathy. According to the Durie and Salmon classification, 16/18 patients were in stage III.

Results: We observed that all the samples studied were CD38+. This antigen was expressed at high density on myeloma PC. CD138 was expressed in all the patients studied; CD117 was significantly expressed in 1/3 of our patients with MM; CD56, instead, was expressed on 11 out of 18 patients. Concerning CD56 expression, the positive cases were more than 50%, on the basis of mean fluorescence intensity (MFI). 4 patients showed a MFI 79.6 (range 42.2-122.8), 5 patients a MFI 521.2 (range 288.7-1042.6). The first group were composed mainly by patients with Iga isotype and by patients with peripheral blood diffusion, thus in presence of circulating PC. Myeloma cell lines, were also characterized by the expression of CD138 and CD38, while they were negative for the other markers.

Conclusions: Since the expression of specific adhesion molecules would explain the migration from the circulating compartment to the bone marrow and viceversa, we suggest that the level of expression of these molecules might regulate the escaping of malignant cells from the marrow environment.

PRE-RNA TRANSCRIPTION/PROCESSING IN LYMPH NODE CELLS FROM PATIENTS WITH LYMPHOMAS

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Introduction: Nucleolar silver staining allows to evaluate quantitatively pre-rRNA transcription/processing in every tested cells by means of a light microscopy.

Methods: Nucleoli and Ag grains (AgNORS) were evaluated in silver-stained lymph node imprints from 32 patients (pts) with Hodgkin's lymphomas (HL), from 31 pts with low- (LG), intermediate- (IM) and high grade (HG) of nonHL, as well as from 32 pts with non-specific lymphadenitis (LA). Imprints were prepared according to our recently published silver-stained technique. U Clin Pathol. Med Pathol 1997, 50:149.

Results: The mean numbers of nucleoli and AgNORS were higher in lymphomas than those in LA although it was correct only with regard of mature cells populations (lymphocytes, monocytes, natural killer lymphocytes and immunoblasts). As shown in the table the highest numbers of nucleoli and AgNORS were revealed in Hodgkin-Reed-Sternberg (HRS) cells from pts with nodular sclerosing (NS) subtype of HL.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Number of nucleoli per nucleolus (number of nucleoli per nucleolus)</th>
<th>Number of AgNORS</th>
<th>Number of nucleoli and AgNORS</th>
<th>Number of nucleoli and AgNORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL NS</td>
<td>1.28 (0.08)</td>
<td>7.85 (0.53)</td>
<td>7.58 (0.58)</td>
<td>10.29 (0.90)</td>
</tr>
<tr>
<td>MC 15</td>
<td>1.21 (0.06)</td>
<td>7.40 (0.42)</td>
<td>7.00 (0.47)</td>
<td>12.64 (0.97)</td>
</tr>
<tr>
<td>non-HL</td>
<td>1.28 (0.91)</td>
<td>7.10 (1.75)</td>
<td>7.29 (0.97)</td>
<td>17.41 (2.97)</td>
</tr>
<tr>
<td>LG 7</td>
<td>1.27 (0.13)</td>
<td>3.08 (0.32)</td>
<td>3.58 (0.37)</td>
<td>6.65 (0.97)</td>
</tr>
<tr>
<td>HG 10</td>
<td>1.36 (0.97)</td>
<td>3.59 (0.34)</td>
<td>3.96 (0.98)</td>
<td>7.55 (1.97)</td>
</tr>
<tr>
<td>LA 32</td>
<td>1.16 (0.31)</td>
<td>2.57 (0.23)</td>
<td>2.75 (0.27)</td>
<td>5.32 (0.97)</td>
</tr>
</tbody>
</table>

Conclusion: The difference in pre-rRNA transcription/processing in different classes of lymphoid cells in lymph node imprints from pts with lymphomas may be related with their maturity, different proliferative potentials and also with elevated production of interleukines, including of transforming growth factor -beta in NS subtype of HL.

BASIC FIBROBLAST GROWTH FACTOR (bFGF) AND BFGR-1 EXPRESSION IN HODGKIN'S LYMPHOMA

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Introduction: It is now clear that angiogenesis and angiogenesis factors are important in the pathogenesis of hematological malignancies. High pretreatment levels of serum basic fibroblast growth factor (bFGF) have been shown to be associated with poor prognosis in patients with non-Hodgkin's lymphoma (NHL). Very little is known about angiogenesis in Hodgkin's lymphoma. This disease is characterized by the presence of Hodgkin and Reed-Sternberg (H-S) cells against a hyperplastic background of reactive cells.

Methods: In this study we performed immunostaining for bFGF and bFGFR-1, and microvessel count (MVC) on 8 lymph nodes taken at diagnosis from Hodgkin's lymphoma patients. Serum bFGF levels was measured by ELISA at diagnosis. Three patients had nodular sclerosis and 5 mixed cellularity. One patient was at stage I, 4 at stage II and 3 at stage III.

Results: The mean serum level of bFGF was 5.4 pg/ml (range 0.4-13). The mean MVC was 70.6 (range 20-140). We found that all 8 patients strongly expressed both bFGF and bFGFR-1 in their H-S cells. In most specimens endothelial cells, polymorphonuclear cells, plasma cells and macrophages stained positive and served as an internal positive control.

Conclusions: Reed-Sternberg and Hodgkin's cells express both bFGF and its receptor, and may respond to bFGF in a paracrine or autocrine fashion, contributing to tumor cell survival.
CORRELATIONS BETWEEN ANGIOGENIC FACTORS (VEGF, bFGF), ENDOSTATIN, MICROVESSEL DENSITY AND CLINICAL OUTCOME: A MULTIVARIATE ANALYSIS IN 82 PATIENTS WITH NON-HODGKIN'S LYMPHOMAS.


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PURPOSE: To determine whether serum levels of angiogenic and antiangiogenic factors and microvesSEL density of lymph nodes were correlated with each other and with various clinical endpoints (overall survival, event-free survival and progression-free survival).

PATIENTS AND METHODS: Patients with non-Hodgkin's lymphoma who had previously been entered in a prospective study carried out between 1994-1998. Blood samples were obtained prior to treatment. Serum bFGF, VEGF and endostatin concentrations were measured using ELISA assays. The microvesSEL density in lymph node biopsy specimens was determined using an anti-vas Willebrand factor antibody and an image analyzing software.

RESULTS: Eighty-five patients treated mainly with ACVPB or CHOP-like regimens with a median follow-up of 62 months. MicrovesSEL density was determined in 28 patients and no correlation was found between this parameter and serum basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and endostatin levels. There was no correlation either between angiogenic factors, endostatin or microvesSEL density and the histological grade or the International Prognostic Index (IP). Complete remission after chemotherapy was significantly correlated with high microvesSEL density and low serum bFGF levels. In the univariate analysis, IP, histological grade and serum bFGF concentrations were prognostic of overall survival. In the multivariate analysis, bFGF values were an independent prognostic factor as well as the IP.

CONCLUSION: bFGF is an independent prognostic factor in patients with non-Hodgkin's lymphoma and might further improve prognostic stratification of patients which is currently based exclusively on IP.

Proliferation, Apoptosis and Intratumoral Vascularity in Multiple Myeloma - Correlation with the Clinical Stage and Cytogetic Grade

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Introduction: Abnormalities involving proliferation, apoptosis and angiogenesis are important in tumorigenesis. The purpose of this study is to comprehensively examine these three biological processes, and their relationship with the clinical stage and cytogetic grade in multiple myeloma (MM).

Methods: Fifty-four newly diagnosed MM cases were studied by immunohistochemistry using bone marrow clot sections. Proliferation and apoptosis were evaluated for the proportion of MM cells (indicated by morphology and CD138 reactivity) positive for the Ki67 antigen and single stranded DNA (dsDNA), respectively. Angiogenesis was evaluated by quantitating the intratumoral microvesSEL density (IMVD) and by assessing the immunoexpression of vascular endothelial growth factor (VEGF).

Results: There were 30 men and 24 women (median age, 65 years; range, 37-84 years). At initial presentation, 15 (28%) were in stage I, 15 (28%) were in stage II, and 24 (44%) in stage III. According to the pathologic stage, there were 13 (24%) in stage I, 31 (57%) in stage II, and 24 (44%) in stage III. There was no significant difference (p=0.03). The mean Ki67, dsDNA and IMVD were 4.4% (0-15%), 0.2% (0-2.8%), and 31.5 (0-63), respectively. Among these two parameters, the only significant correlation was that between Ki67 and IMVD (p=0.0001). Both Ki67 and IMVD also correlated with the clinical stage, cytogetic grade, and VEGF reactivity (p<0.0001). No correlation was found between dsDNA and all of the other parameters.

Conclusions: To conclude, our data suggest that proliferation is associated with angiogenesis in MM. Furthermore, proliferation and angiogenesis, but not apoptosis, may be important in disease progression. Lastly, increased production of VEGF may be one of the contributing factors to the increase in intratumoral vascularity seen in advanced MM.

Evaluation of angiogenesis in non-Hodgkin lymphoma (NHL)

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Introduction: Angiogenesis is required for tumor growth. In solid tumors, the angiogenic activity may regulate the metastatic potential and it often correlates with an unfavorable prognosis. In malignant lymphomas, the role of angiogenesis is as yet unknown.

Aim of the study: To evaluate the pattern of angiogenesis in different subtypes of malignant lymphomas in order to a) establish possible differences between subtypes, b) investigate the occurrence of subtype-specific patterns and c) correlate angiogenic scores to clinical prognostic profiles.

Methods: 34 patients from the Danish Lymphoma Registry, LYFO, were selected for analysis. Pre-therapeutic diagnostic lymph node biopsies from 12 follicular B-cell lymphomas (FL), 12 diffuse large B-cell lymphomas (DLBCL) and 10 peripheral T-cell lymphomas (PTCL, 7 unspecified and 3 anaplastic large cell) were studied.

In all cases, a pre-therapeutic diagnostic lymph node biopsy was investigated. MicrovesSELs in 3-4 µm tissue sections were highlighted by immunohistochemical CD34 staining and scored according to the Chaklasy method. The Chaklasy-counts were then correlated to both histological subtype and pre-therapeutic prognostic (according to the International Prognostic Index (IPI)).

Results: As shown below, we found a significant correlation between histological subtype and Chaklasy-score (p<0.001), detected by Pearson's chi² test.

![Histology Table](image)

A marginally significant, positive correlation between Chaklasy-count and IPI (p<0.04, Spearman R test) was also found.

In PTCL and DLBCL, no correlation was found between Chaklasy-count and response- or relapse-rate (p<0.08 and 0.33, respectively) by preliminary Pearson's chi² test.

Conclusion: PTCL had the highest, DLBCL an intermediate and FL the lowest angiogenic activity. We found a marginally significant correlation between IPI and angiogenesis. These findings need confirmation from larger series of patients to clarify the role of angiogenesis in the biology and prognosis of NHL.

Milt protein expression is found in normal human tissues and different subtypes of non-Hodgkin's lymphoma

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2. Department of Hematology, UCL, Brussels, Belgium

Introduction: The chromosomal translocation t(11;14)(q21;q21) represents the most frequent structural chromosomal abnormality in extranodal marginal zone B-cell lymphomas of the MALT-type and specifically characterizes this distinct subtype of non-Hodgkin's lymphomas (NHL). This translocation leads to a fusion of the annexin inhibitor gene API2 on chromosome 11q21 and the novel MALT/MALT1 gene, which encodes a human paracaspase, on chromosome 18q21. The chimeric protein effectively activates NF-kB, a potential pro-survival signal in B-cells.

Methods and results: To determine the expression levels of the MALT protein semiquantitative western blot analyses were performed on 20 different normal human tissues (Clonectomy) and 27 lymphode biopsies from patients with reactive lymphadenitis (n=8) and different subtypes of NHL (extranodal MALT lymphoma (n=3), follicle center cell lymphoma (n=6), mantle cell lymphoma (n=4), diffuse large cell lymphoma (n=9). Western blot analysis was performed according to standard methods using a polyclonal rabbit antibody directed against the MALT peptide sequence ASP,PRO,LYS,ASP,ALA,ASN,LYS,GLY,THR,PRO,GLU,GLU,THR corresponding to nucleotides 1332 to 1360 from exon 16 of the MALT gene. Data were evaluated by using "NIH-Image" program, taken the testis protein level as reference.

Milt protein expression was found in all 20 human tissues analyzed with the highest expression in plasmoc, fetal brain, ovary, kidney, and cerebellum. Moderate expression levels were detected in testis, lung, trachea, brain, fetal liver, uterus, lymphode, prostate, small intestine, and stomach, whereas low MALT expression was observed in liver, skeletal muscle, heart, spleen, and adrenal gland.

In addition, all lymphode biopsies analyzed showed expression of the MALT protein in high expression levels were found in mantle cell lymphoma, MALT lymphoma, and follicle center cell lymphoma with some variation among the cases.

Conclusion: We conclude that (1) the polyclonal MALT antibody used in this study is a useful tool to detect MALT protein in human tissues, (2) MALT protein is constitutively expressed in human tissues and (3) MALT protein is also expressed in different subtypes of NHL.
Expression of MRDr, MRP, GST-pi and topoisoameras II genes in
non-Hodgkin’s lymphomas
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Introduction: Multidrug resistance of some human cancers, particularly in
relapsed and refractory lymphomas remains a major obstacle to successful
chemotherapy. In this study the expression of MRDr, MRP, GST-pi and
topoisoameras II were evaluated in patient with non-Hodgkin’s lymphomas
(NHL) and reactive hyperplasia.
Methods: The MRDr, MRP, GST-pi and topoisoameras II mRNA levels were
estimated in 29 biopsy samples by semi-quantitative RT-PCR assay using beta-
actin mRNA as an endogenous control. The histologic grade of NHLs was
assigned according to the Working Formula’s classification.
Results: There was no expression of the expression of MRDr, MRP, GST-pi and
topoisoameras II mRNA between NHL and reactive hyperplasia. Among NHL
patients the MRP mRNA level has been significantly higher in patients after
chemotherapy in comparison with untreated ones (p=0.03). Levels of MRP
mRNA were higher in high grade then in low grade lymphomas (p<0.03).

<table>
<thead>
<tr>
<th>Topois</th>
<th>MRP</th>
<th>GST-pi</th>
</tr>
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<tbody>
<tr>
<td>Reactive hyperplasia (n=9)</td>
<td>0.42 ± 0.14</td>
<td>0.03 ± 0.003</td>
</tr>
<tr>
<td>NHL (n=20)</td>
<td>0.35 ± 0.07</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Untreated (n=12)</td>
<td>0.37 ± 0.10</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Treated (n=9)</td>
<td>0.23 ± 0.10</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>High grade (n=5)</td>
<td>0.48 ± 0.16</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Low grade (n=15)</td>
<td>0.32 ± 0.08</td>
<td>0.03 ± 0.008</td>
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Conclusion: These data suggest that MRDr expression is an important
mechanism of drug resistance associated with worse clinical outcome in
relapsed and refractory lymphomas.

GEP ALTERATIONS IN PATIENTS WITH NON HODGKIN’S LYMPHOMA
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Purpose: To study diagnostic alterations in patients with non-Hodgkin’s lymphoma and
to assess diagnostic significance of these findings.
Methods: Formalin fixed and paraffin embedded tissue from 34 patients with large cell
B-NHL, 10 with lymphadenitis chronic (LC), 8 with CTCL and peripheral blood
mononuclear cells from 6 healthy donors were analysed for mutations in codons 12, 13
of K-ras and K-ras genes, c-myc amplification (differential PCR): bcl-2 translocation (FISH)
and mbr (b-c;best), b-cell clonality based on immunoglobulin (Igk/CDR3 and CD22) gene
arrangements and T-cell clonality based on TCR-γ gene rearrangements (multiplex
PCR).

Results: a. Identification of amplified products 10% vertical polyoma-like bands was
used when necessary.

Conclusions: PCR analysis of clonal Igk and TCR γ rearrangements is given priority
when diagnostic assistance is required. This technique has also great potential in
tracking minimal residual disease in lymphomas and leukemias and for monitoring
clonal evolution in acute and chronic lymphocytic leukemias and lymphomas. A
presence of other genetic alterations, that we have detected, could serve as an additional
factor in assessment of tumor biology in patients with NHL.

CYTOGENETIC CHARACTERISATION BY M-FISH AND R-BANDED
KARYOTYPING IN 21 NON-HODGKIN’S LYMPHOMA PATIENTS
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Non-Hodgkins lymphoma (NHL) may have complex karyotypes that
remain uncompletely characterised by conventional cytogenetics alone. We
have thus evaluated multiplex-FISH (M-FISH) as a strategy to unravel one
in this step this complexity (and identify novel recurrent anomalies) 2 confirm
suspected, subtle chromosomal translocations on sometimes ill-defined
chromosomes (13;14;16;22), for example.
R-banded and M-FISH karyotyping (Vysis probes) were performed in
lymph node biopsies from 21 NHL patients (9 follicular lymphoma (FL) and
12 diffuse large cell lymphoma (DLCL)). Nineteen patients were studied at
the time of diagnosis (9 FL and 10 DCLL) and 2 during disease progression.
By R-banding, hypodiploidy was detected in 5 cases (3 FL, 2 DCLL), pseudodiploidy in
(1 FL, 3 DCLL), hyperdiploidy in 11 cases (5 FL, 6 DCLL) and tetraploidy in 1 DCLL patient.
Chromosomal aberrations were more frequent in DCLL than in FL with an average number of changes
per patient of 12 vs 9, respectively. A total of 16 cases showed evidence of
14q32 rearrangement (t(14;16) accounted for the majority (50 FL and 4/12
DCLL). A further 4 14q32 breaks involved 3q27 (1 FL, 1 DCLL) and 8q24 (2 DCLL).
M-FISH confirmed 86 out of a total of 227 chromosomal changes identified by R-banding
(48/81 in FL and 40/146 in DCLL, respectively). A further 36 aberrations were refined by M-FISH
(88/81 in FL and 28/146 in DCLL). M-FISH did not identify t(14;16) in 3/10 patients. Novel aberrations
were identified by M-FISH in all cases except two (1 in FL and 8 in DCLL).
In FL, these included rearrangements of 8p21 (30X), Xq (49X) and Xq (39X).
In DCLL, frequent changes were t12q (512), t4p13 (312), del 8p11 (4/12),
8q11-13 (3/12), 9q10-12 (4/12), 13q21-22 (3/12), 16p12 (4/12).
Except for t12q, these rearrangements have not been previously described in
DCLL. M-FISH thus allows detailed, one step analysis of complex
caryotypes and shows clear potential for identification of novel pathogenic
events in NHL.
INTRODUCTION: 11q deletions are a common abnormality in CLL and are associated with poor prognosis disease. A consensus minimal region of deletion of approximately 1Mb has been identified at 11q32. This region also contains the translocation breakpoint t(11;13)(q23;q12) from a patient with CLL.

Methods: We have constructed a BAC/PAC contig and expression map between D11S300 and D11S1347 to further analyze this region for candidate genes in CLL. The r(11;13)(q23;q12) translocation breakpoint was cloned into a plasmid vector, sequenced and analyzed.

Results: Cloning of the translocation has revealed that the 11q breakpoints is between BOB-1, a B cell specific transcriptional co-activator and PCBP, a member of the BTG family of negative regulators of cell cycle. These genes have been analyzed for REAL time PCR, protein staining where available and expression analysis (with paired buccal and CD19 selected B cell DNA) in 46 patients with CLL. Expression and mutational analysis of PCBP has to date revealed no abnormalities. However, analysis of BOB-1 shows variation in expression among patients with CLL by both real time PCR and protein staining. Heteroduplex analysis by WAVE suggests differences in exon 5 between tumor and constitutional samples of the CLL patients. The translocation results in a promoter and CyC island in the vicinity of BOB-1 which may result in upregulation. BOB-1 is normally associated with germinal center formation and is expressed in cells that are post germinal center in nature. A failure of expression could lead to a lack of VH somatic mutation.

Summary: Although other genes are present within the 11q critical region BOB-1 remains a candidate oncogene for some forms of CLL.

HUMAN HERPESVIRUS 8 (HHV-8) K1 PROTEIN SUPPRESSES BJAB CELL APOPTOSIS INDUCED BY ANTI-FAS ANTIBODY
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Introduction: Human herpesvirus 8 (HHV8) is associated with the development of primary effusion lymphomas (PEL) and Kaposis sarcoma (KS). Virtually all PEL contain HHV-8. K1 is expressed in HHV-8 infected individuals and PEL-derived cell lines express K1 during lytic phase viral replication. K1 encodes for a transmembrane protein containing a short cytosolic tail with an immunoreceptor tyrosine-based activation motif (ITAM). K1 has been shown to aggregate and potentially interact with other membrane receptors. K1 induces activation of NF-kB and its expression in animal models has produces lymphomas. Given its NF-kB induction properties, we anticipated that K1 induces transformation in part by suppression of apoptosis.

Methods: To study the role of HHV8 K1 gene product, we generated K1 stably expressing cell line BJAB-K1 and vector alone cell line BJAB-XS. Anti-Fas antibody was used to stimulate apoptosis and cells were monitored for morphological changes of apoptosis. (1) K1 cDNA was subcloned into retrovirus vector pLXSN and the construct pLXSN-K1 was transfected in packaging cell line PG7 NIH 3T3. Stable cell lines were generated with infectious virions. (2) The K1 expression stable cell line BJAB-K1 and empty vector cell line BJAB-XS were established. (3) Apoptosis of cells was induced by anti-Fas antibody. (4) Apoptosis rates of cell lines were compared with Annexin-V-FITC/PI analysis and DAPI-based nuclear morphological analysis.

Results: (1) The stable cell lines BJAB-K1 and BJAB-XS were confirmed by PCR-based detection of K1 and vector DNA; (2) Fas mediated apoptosis was suppressed by 30%-50% in BJAB-K1 cells versus BJAB-XS in Annexin-V assay. (3) Apoptosis in BJAB-K1 was also suppressed by 51%-10% when analyzed by DAPI staining.

Conclusion: Our data indicates that the expression of HHV8 K1 can suppress Fas-mediated apoptosis. These results suggest that K1 participates in the pathological process through the suppression of apoptosis.

PROSTATE-APOTOPSIS-RESPONSE-4 (PAR-4) ENABLES NEOPLASTIC LYMPHOCYTES TO CIRCUMVENT THE BRIGATION OF CASPASE-3 BY INDUCING DOWN-REGULATION OF cIAP1 AND XIAP AND ACTIVATION OF CASPASE-6, -7 AND -9
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In previous studies we demonstrated the deregulated expression of Prostate-apoptosis-response-4 (Par-4) in neoplastic cells of acute and chronic lymphocytic leukemia, as well as its proapoptotic effect upon chemotherapy treatment, an effect caused by down-regulation of antiapoptotic Bcl-2 family members and subsequent premature and enhanced disruption of mitochondrial membrane potential. Consequently, we now evaluated the influence of Par-4 on the down-stream events of apoptosis. Molecular cloning of Par-4 cDNA from thyroid cancer cell lines Jurkat was transfected with Par-4 cDNA and stably expressing cell clones were generated by G418 selection under limiting dilution conditions. Par-4 overexpressing clones were assessed in comparison to mock-transfected clones without and with incubation with the anthracidine doxorubicin. As demonstrated by western blot analysis Par-4 overexpression alone was sufficient to induce Parp-cleavage in all transfected cell clones, an effect not detectable in Par-4 negative clones. Upon induction of apoptosis with doxorubicin Parp-cleavage activity increased in Par-4 overexpressing clones as compared to mock clones, as expected accompanied by an augmented rate of apoptosis. Noteworthy, whereas broad-spectrum caspase inhibition by ZVAD-fmk completely abrogated Parp-cleavage in Par-4 positive as well as negative cell clones, specific inhibition of caspase-3 by DEVDO-cholesterol abrogated Parp-cleavage in Par-4 negative clones only, i.e. Par-4 expressing clones retained Parp-cleavage activity despite absence caspase-3 activity. To clarify this persistent Parp-cleavage activity of caspase-7 and 9, in presence of DEVD-cholesterol was observed, demonstrating an increased activity of both enzymes in Par-4 expressing clones as compared to mock clones. In conclusion our results outline a model of caspase activation explaining the sensitivity of neoplastic lymphocytes to apoptosis stimuli upon Par-4 expression. We thus demonstrate, that the rate of apoptosis increases due to elevated caspase-3 activity, and that overexpression of Par-4 enables neoplastic lymphocytes to circumvent the abrogation of caspase-3 activity by inducing down-regulation of cIAP1 and XIAP, as well as activation of caspase-6, -7 and -9.
SOLUBLE CYTOKINES CD30, IL-2R AND IL-10 AS MARKERS OF MATURE AND TRANSFORMED LYMPHOID MALIGNANCIES
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Introduction: Soluble cytokines can be used as markers of different forms of lymphoid malignancies (Hodgkin’s disease, diffuse B-large cell lymphoma, “gray zone” lymphomas) as we showed in the Therapeutic Archives 2001, 4, 45-51. It was necessary to find some correlation between quantity of these cytokines and nosological form of these malignancies.

Methods: Serum cytokine concentrations were measured by ELISA several times in the courses of polychemotherapy in 245 samples of 140 pts - Hodgkin’s disease 45 pts, diffuse B-large cell, anaplastic large cell and mediastinal B-cell lymphomas - 47 pts, mature cell and transformed lymphoid malignancies - 48 (CLL 30 pts, mantle cell lymphoma 10, follicular lymphomas 3, marginal cell lymphomas 5) pts.

Results: in CLL pts serum IL-2R levels are always high and do not correlate with low serum CD30 levels - the main difference from lymphosarcoma. In transformed CLL the relative levels of cytokines CD30 and IL-2R are the same as in lymphosarcomas and correlates with the response to chemotherapy. Serum IL-10 level in CLL pts was always lower than threshold concentration for lymphosarcomas. In mantle cell lymphoma sera there were two variants of cytokines levels: 1) high level of CD30 which did not decrease during chemotherapy courses and high level of IL-10, 2) low level of CD30 which decreases during chemotherapy. Survival rate without remission less than 2 yrs. In both situations serum IL-2R level correlates with serum CD30 level.

Conclusion: Serum cytokines CD30, IL-2R and IL-10 are important additional markers for diagnostic and prognostic evaluation of malignant B-lymphomas.

TRAIL/Apo2L Induces Apoptosis of CD30+ Cell Lines Independently of CD30 Activation. P. Fiumara, V. Smeli, Y. Li, A. Mukhopadhyay, M. Younes, M. Andreff, F. Cahnallia, A. Carbon, B. Aggarwal, and Y. Younes. M.D. Anderson Cancer Center; Baylor College of Medicine, Houston, TX, USA; and, Istituto Nazionale Tumori, Aviano, Italy.

Introduction: TRAIL (Apo-2L) is a potent death protein belonging to the TNF family. In addition to mediating T cell and natural-killer cell cytotoxicity, TRAIL has been shown to induce the death of a wide range of primary tumors and cancer cell lines. CD30 has been shown to enhance the survival of the malignant Hodgkin and Reed-Sternberg (HRS) cells of Hodgkin disease (HD), or to induce cell death of non-HD derived CD30+ cell lines. The expression of TRAIL receptors, the activity of TRAIL, and the interaction between TRAIL and CD30 ligand (CD30L) in HD are not known.

Methods: we studied TRAIL activity in four well-defined HD cell lines (3 CD30+ and one CD30-) and compared it to TRAIL activity in 3 CD30+ lymphoid cell lines not derived from HD. We also determined the modulatory effect of CD30 ligand (CD30L) on TRAIL-induced cell death in these cell lines.

Results: TRAIL, effectively killed a CD30-negative HD cell line (HD-MY2), and had either no or little effect on the remaining HD cell lines (HDLM-2, L-428, and KM-HD).

The CD30+ phenotype did not confer resistance to TRAIL as other CD30+ cell lines were highly sensitive to TRAIL. Resistance to TRAIL was not related to differences in the expression of TRAIL receptors, FADD, SODDO, procaspase-8, 10, 9, 3, or inhibitors of apoptosis proteins (IAPs). Three of the 4 TRAIL-sensitive cell lines had no detectable levels of the anti-apoptotic protein FLIP, whereas all 3 TRAIL-resistant cell lines expressed high levels of FLIP. Activation of CD30 by recombinant CD30L did not inhibit TRAIL-induced cell death and did not regulate intracellular anti-apoptotic proteins in the TRAIL-sensitive CD30+ cell lines, neither potentiating the activity of TRAIL in the HD cell lines.

Conclusions: Collectively, these data suggest that sensitivity to TRAIL is independent of CD30 activation, and that TRAIL as a single agent may have a potential therapeutic value in CD30+ lymphoid tumors that are not derived from HD.

THE EFFECT OF TGFBeta1 ON FL- AND MCL-DERIVED CELL LINES.
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Introduction: Lymphomagenesis is a multi-step process associated with the activation of neopigenes and the inactivation of tumor suppressor genes. The products of such regulatory genes are mostly involved in cell cycle machinery, apoptotic cascade, cell-cell contacts, and signal transduction pathways including the anti-apoptotic cell signals. The aim of our project was to analyze the sensitivity of three different NHL cell lines to the inhibitory action of TGFBeta1 and to study the composition/activity of catalytic cytokine- dependent kinase (CDK) complexes.

Materials and Methods: MCL-derived cell lines Rec-1 and Granta-519, and FL-derived cell line DoHH2 were used for the study. The level of G2-phase and S-phase cyclins, CDKs, CDK inhibitors (INK and CDK/KIP families), and the level of CREBBATF transcription factors was mostly studied both by polyclonal chain reaction (PCR) and by immunoblotting. Kinase activity of CDK complexes was tested against RB-GST substrate in the presence [32P]ATP. The binding affinity of CREBBATF family proteins towards the cyclin A promoter sequences was analyzed by Gel Mobility Shift Assay. The effect of TGFBeta1 on the cell cycle was evaluated by flow cytometry.

Results: TGFBeta1 treatment (10 ng/ml) led to significant growth arrest of DoHH2 cells while both MCL-derived cell lines proved to be TGFBeta1-resistant. Surprisingly, all three malignant cell lines expressed high level of CDK inhibitors. The expression of cyclins and CDKs was not modulated by the TGFBeta1 treatment, except the level of cyclin A. The kinase activity of CDK2 and CDK6 was inhibited, CDK4 activity was notably reduced. To elucidate the absence of cyclin A in TGFBeta1-treated DoHH2 cells, we studied the expression/activity of CREBBATF transcription factors. The total level of CREBBATF transcription factors. The total level of CREBBATF-1, ATP-2, and ATP-3 was notably reduced. Moreover, CREBBATF-1 was phosphorylated during the treatment. The binding activity of CREBBATF factors to the CRE region of cyclin A promoter was then significantly reduced.

Conclusions: We analyzed the ability of TGFBeta1 to induce growth arrest in three malignant NHL cell lines. In TGFBeta1-sensitive cell line, the anti-proliferative effect of TGFBeta1 was associated with the absence of cyclin A molecules and subsequently with the inhibition of cyclin A-related CDK2 activity. The expression of cyclin A gene expression was downregulated by the absence of stimulatory CREBBATF transcription dimers.

THE GENETICS OF LOW-MALIGNANT B-CELL DISEASES
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The chronic B-cell lymphoproliferative disorders with abnormal circulating cells include chronic lymphatic leukemia (CLL), hairy cell leukemia (HCL), and splenic lymphoma with villous lymphocytes (SLVL). We speculate that the clinically observed differences between the diseases reflect differences in the genetic background. Unfortunately, in all three diseases the malignant cells have too low a spontaneous mitotic index for efficient standard cytogenetic analysis. We have therefore employed Comparative Genomic Hybridization (CGH) for the detection of characteristic chromosomal gains and losses in samples from 72 patients with one of the above-mentioned diseases (26 patients with CLL, 28 with HCL, and 18 with SLVL). The percentage of malignant cells in the samples was first evaluated by flow cytometry, and if the fraction of malignant cells in the original sample constituted <50%, an immuno-magnetic-B-cell purification was performed. For all patients included in this study the fraction of malignant cells in the final sample constituted >50%. CGH was performed basically as described by Kallioniemi et al. (1992)[1]. CLL was most frequently associated with loss of 8p11-pter, 11q22-23, and 17p12-ppter, and gain of 12q15-ppter. HCL was most frequently associated with loss of 7q21-35, and gain of 5q11-ppter. SLVL was most frequently associated with loss of 6q23 and 7q31, and gain of 3q25-qter and 12q31-qter. The three diseases are thus rather distinct in their patterns of gains and losses. We are currently testing and elaborating these findings in an interphase Fluorescence In Situ Hybridization (FISH) study of the most frequent abnormalities. This study will also include the samples that did not fulfill our purity criteria for CGH.

LACK OF HER2/NEU-OVEREXPRESSION IN NON-HODGKIN'S LYMPHOMA
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Introduction: In case reports, overexpression of HER2/ neu is suggested not only in solid tumors but also in Non-Hodgkin's lymphoma (NHL), especially diffuse large-cell lymphoma [Minebesa et al., 1993; Cheung et al., 1999].

Methods: From a serum bank of a hematological department, we received serum samples from 87 consecutive unsolicited patients with NHL and tested for the shedded antigens of HER2/neu using the Oncogene Science® ELISA assay (Cambridge, MA, USA). Among these lymphoma patients, the paraffin-embedded lymph-node specimens of 25 cases with diffuse large-cell lymphoma (DLCL) were stained with the HER2/neu DAKO HercepTest™.

Results: In 87 NHL patients, the percentage of HER2/neu ranged from 3.6 – 244.1 ng/ml (median 7.8 ng/ml). Only 2 patients showed a marginal or increased HER2/neu level with 15 ng/ml (which is the upper limit of normal) and 244.1 ng/ml. No patient with DLCL showed HER2/neu overexpression by immunohistochimistry of the lymph-node. The paraffin block of the one patient with a very high HER2/neu serum level was stained for HER2/neu overexpression. In this patient, suffering from a high-grade T-cell NHL, no staining could be found.

Conclusions: HER2/neu is not overexpressed in NHL and especially not in DLCL using a standardized immunohistochemistry technique with complementary serum testing.

EPIDERMAL GROWTH FACTOR RELAY SIGNAL TRANSDUCTION IN MITOGENIC PROLIFERATION OF BREAST CANCER CELLS
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Introduction: Mitogenic growth factors of the EGF-family are known to induce mitogenic proliferation of breast cancer cells. An essential mediator of mitogenic growth factor signalling in breast cancer cells is the G174 and G181 serum inhibitors. G174 and G181 (212 ng/ml) had each at least a two-fold higher selectivity for stimulatory against the EGF receptor than for the G174 and G181 receptor.

Methods: The inhibition of apoptosis was quantified by measuring the number of cell survival and the number of cell proliferation by a cell counting.

Results: Cell proliferation was strongly related to ALK status: ALK positive ALC1 had significantly higher levels of active caspase 3, while high expression of the anti-apoptotic proteins Bcl-2 and Bcl-9 was almost completely restricted to ALK negative cases.

Conclusions: High numbers of active caspase 3 positive tumor cells predict a high favorable outcome. High numbers of Bcl-2 and Bcl-9 positive tumor cells were found to indicate unfavorable prognosis. This prognostic effect was strongly related to ALK status: ALK positive ALC1 had significantly higher levels of active caspase 3, while high expression of the anti-apoptotic proteins Bcl-2 and Bcl-9 was almost completely restricted to ALK-negative cases.

ABERRANT DNA METHYLATION OF P57KIP2 GENE IN THE PROMOTER REGION IN LYMPHOMA MALIGNANCIES OF B-CELL PHENOTYPE
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Introduction: p57KIP2, a kind of cyclin-dependent kinase (CDK) inhibitor, has been thought to be a good candidate for a tumor suppressor gene (TSG). The purpose of this study was to examine the possibilities of p57KIP2 gene as a TSG.

Materials and Methods: 19 DLBCL, 18 FL, 18 CLL, 26 MM, 51 ATL, 52 MDS, 14 AML, 6 ALL, 2 reactive lymphadenitis, 1 normal PBMNCs, and 17 hematolymphoid cell lines were analyzed. The expression of p57KIP2 was examined by RT-PCR. Promoter DNA methylation was analyzed by methylating-specific PCR and bisulfite sequencing. Mutation of p57KIP2 was examined by PCR-SSCP analysis.

Results: Expression of p57KIP2 gene was diminished or absent in various kinds of hematological cell lines. This expression was restored by treating cell lines with 5-aza-2'-deoxycytidine. Bisulfite sequencing analysis of its promoter region showed that cell lines with no expression of p57KIP2 gene had complete DNA methylation. DNA methylation of this region itself is thought to be an aberrant alteration, since normal peripheral blood mononuclear cells and reactive lymphadenitis did not show any DNA methylation. By methylating specific PCR analysis, we found frequent DNA methylation of p57KIP2 gene in primary DLBCL (54.9%) and follicular B cell lymphoma (54.0%) as well as in secondary MDS (20.0%) and adult T cell leukemia (20.0%). We could not find any mutations of the gene in 31 DLBCL by the PCR-SSCP analysis.

Conclusions: The aberrant DNA methylation of p57KIP2 gene at its promoter region might be one of the mechanisms of silencing p57KIP2 gene expression. p57KIP2 was frequently inactivated by promoter DNA methylation in B-cell malignancies. These findings directly indicate the profile of p57KIP2 gene as a TSG candidate.

ANALYSIS OF CYTOGENETICS ON SERIAL BIOPSY SPECIMENS AND CORRELATION WITH CLINICAL OUTCOME IN PATIENTS WITH FOLLICULAR LYMPHOMA FROM BRITISH COLUMBIA
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Introduction: Follicular lymphoma is associated with cytogenetic abnormalities, the most common being t(14;18)(q32;q21) or its variants. Previous studies have suggested that changes such as t(14;18), +1, +17p, +18q, +12 may be associated with aggressive course or short survival. However, few studies have examined serial cytogenetics in a group of patients with a uniform diagnosis of t(14;18) positive follicular lymphoma.

Methods: We identified 18 patients with follicular lymphoma and t(14;18)(q32;q21) or the variant t(14;18)(ql1q21), each had at least two biopsy specimens for cytogenetic analysis at least 8 months apart. Patients were frequently seen by their primary care provider. None of the patients had t(14;18) transformation to aggressive histology lymphoma, confirmed by excisional biopsy or cytology in eight. Two transformations involved t(14;24) and were Burkitt-like by histology and seven were diffuse large B cell lymphoma. Eight patients died of lymphoma and ten are alive at a median follow-up of 82 (range 27-220) months.

Results: The presence of +5 at any point portended significantly shorter progression free survival (FFS, median 1.6y with 6q- vs. 5.6y, p=0.003). The presence of 3p- at any point appeared to predict better overall survival (OS, median 7.0y with 3p- vs. not reached at 10.5y, p=0.3). Several other factors were analyzed for their association with clinical outcome and were not significant for either FFS or OS, including transformation to higher grade histology, the presence at any point of t(7 or 1p), the acquisition of t(4;24), t(3q), marker chromosomes, 5p-, tetrasomy or 5p- or gain of a minimal number (from 0 to 1+1v10+1v10) total changes or -1 to +1v10+1v10 imbalances, and the presence or acquisition of early vs. late changes (Horman et al., in press; +7, +8, del(17q), +1q, -6q, -X, +12, for early changes; -17q, -17p, -1q, -15, +9, +12, for late changes).

Conclusions: In this series, we were unable to detect serial cytogenetics changes predictive of clinical outcome. However the presence on any specimen of 6q- was associated with shorter FFS, and 3p- appeared favorable for OS. Small numbers may have limited the ability to detect other associations, for example only 2 patients harbored alterations in t(14;24), which is known to portend for poor outcome. Larger series of serial biopsies may reveal progressive cytogenetic changes associated with clinical outcome in patients with follicular lymphoma.
NUCLEAR EXPRESSION OF NFκB IS NOT RESTRICTED TO NON-MALIGNANT CENTRE DIFFUSE LARGE B CELL LYMPHOMA
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Diffuse large B cell lymphomas (DLBCL) have been sub-classified as germinal centre (GC) and activated B cell (ABC) types using gene expression profiling, and this has been confirmed in some studies using immunohistochemical analysis. More recently, NFκB mRNA has been shown to be increased in ABC, but not in GC DLBCL. The aim of this study was to evaluate the expression of NFκB using immunocytochemistry in a series of DLBCLs, and to correlate expression with clinical and biological variables and patient outcome. Paraflin-embedded biopsies from 135 cases of primary nodal DLBCLs were stained for NFκB using the Delta Biolabs DB033 NFκB p65 (C20), and heat-modified antigen retrieval.

Reactive T-cells strongly expressed cytostatic or nuclear NFκB, and served as an internal control. 86/135 (64%) DLBCLs expressed NFκB in the tumour cell cytoplasm, and 26/135 (19%) cases also had occasional tumour cells with nuclear localisation of NFκB in addition to cytostatic staining. In a further 23/135 (17%) of cases tumour cell NFκB expression was exclusively nuclear. The DLBCLs used in this study have been previously classified according to clinical and biological variables, including IPI, BCL2 expression, t(14;18), and GC phenotype. 15/52 (29%) GC cases and 34/83 (41%) non-GC DLBCLs had nuclear localisation of NFκB in at least a proportion of the tumour cells. Nuclear NFκB was not significantly associated with GC phenotype or any other variable tested using Chi squared analysis. Although there was a trend towards a less favourable outcome, (33% 5-year OS in the nuclear NFκB group compared to 52% in cases with cytostatic expression) statistical significance was not reached (Log Rank, p=0.1).

Similarly, no effect on outcome was seen when examining NFκB status in the individual IPI categories or in the context of GC status.

The results of this study have shown that differential NFκB protein expression is not restricted to non-GC DLBCL, and highlights the lack of concordance of results obtained by mRNA expression profiling and immunocytochemistry. Although there was no significant effect on outcome based on NFκB expression, this group may benefit from treatment with proteosome inhibitors.

CLONAL DEVELOPMENT OF A PROGRESSIVE MANTEL CELL LYMPHOMA STUDIED BY COMPARATIVE GENOMIC HYBRIDIZATION
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Introduction: Mantle cell lymphoma (MCL) is an entity of B-cell derived non-Hodgkin lymphoma which typically shows an aggressive clinical course. Cytogenetically, MCL is characterised by the translocation t(11;14)(q13;q32) which leads to over-expression of cyclin D1, a cell cycle regulator normally not expressed in B-cells. However, recent evidences suggest that additional oncogenic factors are required for MCL development. Characterization of additional chromosomal aberrations are expected to contribute to the understanding of the development and progression of the disease. In this case report, we applied the comparative genomic hybridization (CGH) method which detects gains or losses of chromosomal regions, to follow the pattern of chromosomal aberrations during tumor progression in a patient with MCL.

Materials and Methods: CGH was performed using DNA extracted from the diagnostic tumor sample and three relapses from a case with a blastoid variant of MCL. Clonal relationship of the samples was investigated by single stranded conformation polymorphism (SSCP) analysis.

Results and Discussion: The clonal relatedness of the tumors was demonstrated by identical Ig heavy chain (IgH) gene rearrangements detected by SSCP. The clonality was also supported by the CGH profiles. All samples shared the common alteration fusions of 6q, 9p and 14q and gains of 5q, 9q, 12p and 13q, suggesting that they were early events in the tumor progression. In relapse 1, loss of 8p was identified while relapse 2 and 3 gained 7p and X. In addition, relapse 3 gained chromosomes 3 and 20. Taken together, the findings suggest that relapse 2 and 3 developed from the diagnostic tumor sample, while relapse 1 represents a separate lineage of tumor progression originating directly from a postulated primary tumor cell.

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Diagnostic aid of molecular genetic and immuno-flow cytometry in mantle cell lymphoma.
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Background: Mantle cell lymphoma (MCL) is relatively newly defined form of non-Hodgkin's lymphoma (NHL). It represents about 6 to 8% of all NHL. This lymphoma and its morphological variants belong to frequently misdiagnosed lymphoma entities. We attempted to describe the clinical and laboratory features, and to analyze a contribution of modern laboratory methods to diagnostic accuracy in MCL patients.

Patients and methods: Clinical and laboratory data, peripheral blood and bone marrow analysis including genetic analysis, and lymph node biopsy from 56 MCL patients, who were diagnosed and treated in our institution between 1995-2001, were evaluated.

Results: The patients were predominantly male (61%). Median age was 63 years. 96% of all patients had advanced stage disease mostly with bone marrow involvement (92%). Peripheral blood involvement with lymphocytosis (≥4,0×10^7/l) was detected in 61% of cases. Bone marrow aspiration and immuno-flow cytometric analysis were performed in all 56 patients. The presence of monosomy MCL cell population with characteristic phenotype (strong sMlg+, CD5+, CD19+, CD20+, CD23-) was detected in 50 cases (89%). A typical t(11;14) was found by FISH analysis in 13 of 24 cases (54%) and by IGH rearrangement in 8 of 24 studied patients (24%).

Wrong initial diagnosis based on lymph node histology alone was primarily presented in 40% cases. The final diagnosis of MCL in these pts. was done after revision by expert pathologist and with the knowledge of the tumor immunophenotype and/or genetic and molecular genetic changes.

Conclusions: MCL represents relatively frequent diagnostic problem. Some sophisticated laboratory methods (or their combination) can improve diagnostic FISH and immuno-flow cytometry seem to be the most promising and helpful ones with high diagnostic and differential diagnostic benefit.

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ISOTYPE-SWITCHED IMMUNOGLOBULIN GENES WITH A HIGH LOAD OF SOMATIC HYPERMUTATION AND LACK OF ONGOING MUTATIONAL ACTIVITY ARE PREVALENT IN MEDIASTINAL B CELL LYMPHOMA
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Primary mediastinal B cell lymphoma (PMBL) is a subtype of diffuse large B cell lymphoma (DLBCL) with characteristic clinical, histomorphologic, immunophenotypical, and genetic features. Unlike other B cell lymphomas, PMBL has not yet been the subject of comprehensive molecular studies on the rearranged immunoglobulin (Ig) gene. Such investigations have proved essential to obtain information about the differentiation stage of the lymphomagenic B cell. In the present study, we analyzed the clonally rearranged Ig heavy chain gene of 13 PMBL cases by polymerase chain reaction (PCR) in conjunction with cloning and DNA sequencing. Twelve out of 13 rearrangements were potentially functional. All clonally rearranged Ig genes bore a high load of somatic mutation (average: 13.0%) which appeared to be selected for a functional antibody in the majority of cases. The comparison of cloned PCR products revealed no evidence of ongoing mutation of the Ig variable gene. Using reverse-transcribe PCR, we detected lymphoma-specific Ig transcripts in 8 out of 13 cases, all of which were of the post-switched type, whereas Ig protein expression was undetectable in all but one case. A PMBL cell line, MedB-1, generated from an IgG2a parental tumor, constitutively expressed IgG1 protein in a subset of cells, which was moderately suppressed by IL-4 and up-regulated in the presence of dexamethasone. PMBL is thus characterized by a heavily mutated, class-switched Ig gene without evidence of ongoing mutational activity. Moreover, our data indirectly suggest that regulation by extrinsic signals contributes to the Ig phenotype of PMBL.

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Constitutive NFκB/Rel and B-3-Activities in Cutaneous T Cell Lymphomas (CTCLs) are Regulated by a Tyrosine Kinase / Ikk Pathway and Direct the Expression of a CTCL Disease Progression Marker U. Dobriner, J. Kamaretsos, J-Z. Qin, R. Duamer, G. Borg, and F.P. Nentwich
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Introduction: CTCL cells contain NFκB proteins that bind constitutively to their DNA recognition motifs. The expression of the IL-2R alpha chain, which protects against apoptosis, may be regulated by the expression of the IL-2R alpha chain. Methods: The expression of NFκB inhibitors on the DNA binding of NFκB proteins were studied by electrophoretic mobility shift assays (EMSA). The expression of the IL-2R alpha chain was determined by Western blotting. As NFκB inhibitors we used deoxymethosine, which directly interacts with NFκB, sodium salicylate, which inhibits IκK (inhibitor of IKK), which initiates the degradation of the NFκB inhibitor IκB by serine phosphorylation, and the src-type tyrosine kinase inhibitors herbimycin A and P2P. Results: The EMSA and Western blot experiments show that all four NFκB inhibitors down-regulate NFκB DNA-binding and the expression of the soluble form of the IL-2R alpha chain in CTCL cell lines. At higher application times sodium salicylate and herbimycin A cause apoptosis of CTCL cells. Conclusion: Since all four reagents inhibit NFκB, we conclude that NFκB is activated by a src-type tyrosine kinase -> IκK -> NFκB pathway in CTCL cells. The down regulation of NFκB was sufficient to repress IL-2R alpha chain expression. Interestingly, herbimycin A and sodium salicylate may also act on other signaling pathways that regulate cell survival. P2P is a specific inhibitor of the T cell receptor associated, src-type tyrosine kinases Lck and Fyn, whereas herbimycin A is a more general inhibitor of src-type tyrosine kinases. Thus Lck and Fyn may constitute a constitutively active NFκB in CTCL cells, but another src-type tyrosine kinase that is susceptible to herbimycin A, but not P2P, must be necessary for the survival of CTCL cells. The findings that sodium salicylate down-regulates NFκB and that IκK concentrations are low indicate that IκK is involved in the constitutive activation of NFκB in CTCL cells. Herbimycin A and sodium salicylate may be prototypes for reagents that kill CTCL cells and make them more susceptible to NK attack.

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DENDRITIC CELLS AND ADULT T-CELL LEUKEMIA/LYMPHOMA
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Introduction: Adult T-cell leukemia/lymphoma (ATLL) is a hematological neoplasm of mature T cells caused by Human T lymphotropic virus type 1 (HTLV-I) infection. It still remains unknown why only small portion of HTLV-I carriers allow the monoclonal proliferation of tumor cells (ATLL cells).

Methods: We treated 10 patients with ATLL (4 acute type and 6 lymphoma-type) for the last two years (1999-2001). Blood specimens (6 lymph nodes and 1 spleen) from them were analyzed by Southern blotting and immunostaining in order to clarify a interplay between dendritic cells and ATLL cells. In addition, dendritic cells were derived from PBMC from a HTLV-I carrier and the co-culture with PHA-PBMC was performed.

Results: Southern blot analysis showed monoclonal/pleural proliferation of HTLV-I infected cells (ATLL cells) in each lesion. Fas positive cells were detected in LN and spleen of all patients. Some of them were also positive for CD30 and/or CD40. Each lesion was composed of Fas positive cells surrounded by ATLL cells. Dendritic cells were identified by the morphological feature.

Characteristics of dendritic cells in LN and spleen from ATLL

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DC derived from monocyte suppressed the proliferation of autologous PHA-PBMC in carrier state.

Conclusion: These results suggested that dendritic cells play an important role for monomolecular proliferation of ATLL cells and development of ATLL.

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THE CHIMERIC TRANSCRIPT NPM-ALK IS A USEFUL MARKER FOR THE STUDY OF MINIMAL RESIDUAL DISEASE IN ALCCL
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Introduction: Anaplastic Large Cell Lymphomas (ALCL) represent approximately 15% of all pediatric non-Hodgkin's lymphomas (NHL). They are characterized by the reciprocal chromosomal translocation t(2;5)(p23;q35) in about 60-70% of the cases. The genes involved are the nucleophosmin (NPM) gene on chromosome 5, which encodes a nucleolar protein relevant for the assembly and function of ribosomes, and the Anaplastic Lymphoma Associated Kinase (ALK) gene which encodes a tyrosine kinase receptor, the function of which is not completely known, yet.

Methods: The fusion transcript NPM-ALK can be detected by RT-PCR using NPM and ALK specific primers. We established an RT-PCR assay which can detect 10-3 tumor cells and evaluated a series of patients enrolled in the EJOP protocols NHL97 and ALCL99.

Results: 32 biopsies from children affected by ALCCL were studied. In 23 patients we also obtained bone marrow (BM) aspirate at diagnosis which made it possible to study the minimal disseminated disease. Whenever possible the response kinetics to treatment of minimal residual disease (MRD) in BM was determined.

Among the 32 patients, 24 were positive for the NPM-ALK transcript by RT-PCR. 22 of them were studied for minimal BM infiltration at diagnosis and 11 scored positive. Six of them were negative both by microscopic aid immunophenotypic assays. MRD study was performed in 9 patients, 7 in first diagnosis and 2 at relapse. Among the first group of children, 1 became negative after the first week of therapy, 2 at stop therapy, 2 showed clearance of BM disease, but their MRD recurred while still on therapy, and 2 did not reach a BM negative status as determined by RT-PCR. Of the 2 relapsed patients, one became negative after completing the first chemotherapy cycle and 1 only after intensification therapy and allogeneic BM transplantation.

Conclusions: RT-PCR for the NPM-ALK transcript represents an important tool for the characterization of ALCCL at diagnosis and for the MRD studies in patients with BM infiltration at tumor onset. Response to treatment of MRD seems to be rather variable and not so rapid as observed in other hematopoietic malignancies. The study will continue to determine, on a larger series of patients, the prognostic value of MRD in ALCCL.

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Introduction: The chromosomal translocation 8(14)(q24;q32) represents a specific marker for Burkitt's lymphomas (BL). About 75% of BL possess this chromosomal aberration that involves the c-myc oncogene on chromosome 8 and its cellular homolog heavy-chain (IGH) locus on chromosome 14. Methods: Because the translocation does not produce a fusion gene and because of the variability of the breakpoints on both chromosomes, we established a long-distance PCR assay which can amplify up to 15-20 Kb sequences making it possible to detect the 8(14) at the genomic level. The assay is based on 4 separate PCR reactions in which one primer complementary to the first exon of the c-myc gene is used with one of four primers for the IGH locus (1 for the JH region and 1 for each of the 3 constant regions). Results: The sensitivity of the LD-PCR determined by limiting dilutions of BL cells in control DNA was determined to be about 10% of BL patients and found a specific PCR product in 51 of them. Based on the different IGH primers used, we assessed the involvement of the Cj region in 19 cases, Cy in 8, Cc in 15 and JH in 7. Among the last 8 cases, 7 showed a positive standard morphologic and immunophenotypic analysis. In 8 of these patients we also conducted a study of minimal residual disease and determined the responses to treatment. Conclusions: The LD-PCR for 8(14) can be used to complement the histologic diagnosis of BL and represents an important tool for the study of minimal disseminated and minimal residual disease. The application of this technique to large series of patients within homogeneous treatment protocols will allow to determine the prognostic value of minimal disseminated disease and of its response kinetics in BL.


1N5EUBA EPI 9006, Medicine, University, product which suggests that an internal deletion occurred within the IGH locus, although studies to define this finding are in progress. Among the 51 positive patients we studied both the tumor and bone marrow at diagnosis in 36; in 12 patients BM was positive by LD-PCR, whereas only 4 were positive at the standard morphologic and immunophenotypic analysis. In 8 of these patients we also conducted a study of minimal residual disease and determined the responses to treatment. Conclusions: The LD-PCR for 8(14) can be used to complement the histologic diagnosis of BL and represents an important tool for the study of minimal disseminated and minimal residual disease. The application of this technique to large series of patients within homogeneous treatment protocols will allow to determine the prognostic value of minimal disseminated disease and of its response kinetics in BL.


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Abnormalities of the P35 gene at 17p13 are common in many cancers, and are often associated with disease progression, transformation and resistance to treatment. We have analysed a series of 160 cases of B-lymphoproliferative disorders (78 peripheral bloods (PB), 48 bone marrow aspirates (BMA) and 34 lymph nodes (LN)) for the incidence of 17p13 deletions using interphase FISH (cosmid kindly provided by Dr V Ross, Salisbury, UK). 44/160 cases were unsuitable for analysis, mostly due to low peripheral blood B cell counts. 8/116 cases had a demonstrable 17p13 deletion in 25% of nuclei and were classified as suspicious, and follow-up samples have been requested. Of the remaining 108 samples: 31 (28%) had a 17p13 deletion in 5-75% of the cells. The deletions were demonstrated in 14/68 (20%) B-CLL, 3/30 (10%) (11;14) positive mantle cell lymphoma (MCL), 1/8 (12.5%) marginal zone lymphoma (MZL), 9/14 (64%) 4;18, or variant, positive follicular lymphoma (FL) and 6/8 (75%) diffuse large B cell lymphoma (DLBCL). Of the cases with a demonstrable 17p13 deletion, immunocytochemistry for P53 and P21 proteins was carried out in 24 cases, where histological samples were available. 15/24 (63%) cases expressed P53 protein and of these, 13/15 did not express P21. The majority of cases analysed, including those with a 17p13 deletion (12/14 B-CLL, 5/6 DLBCL, 5/9 FL and the MCL and MZL), were presentation samples. 9/14 (64%) CLL cases had deleted 17p13 in addition to deleted 10q24 and/or trisomy 12. Deletion of 17p13 is a common feature of presentation B-lymphoproliferative disorders, particularly of FLCL and DLBCL type. Of the cases with P53 expression, the majority of cases lacked expression of P21, suggesting mutation of the remaining allele. It may be implied that loss of one allele may increase the risk of mutation of the other allele and in this context; 17p13 deletion would be expected to be prognostically relevant across the range of B-lymphoproliferative disorders.
IMMUNOCHEMISTICAL AND WESTERN BLOTTING ANALYSIS FOR THE EXPRESSION OF CASPASA 3 IN MONONUCLEAR CELLS OF THE PERIPHERAL BLOOD IN CLL PATIENTS

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Introduction: B cell chronic lymphocytic leukemia (B-CLL) is characterised with the progressive accumulation of mature B lymphocytes more than disturbances in the proliferation itself. The accumulation of the neoplastic transformed B lymphocytes might be due to uncharacterised defects in the apoptosis. The execution phase of this process is the activation of the caspasa 3 protein, same as other enzymes from caspasa family. Recent investigations shows that caspasa 3, 7 and 8, but not caspasa 2, are involved in the execution phase of apoptosis B-CLL cells.

Methods: By using Western blotting and immunochemistical method APAAP we have analyzed the expression of caspasa 3 in the peripheral blood samples of the patients (pts) with CLL. We have analyzed 20 pts who achieved Chloramphenicol and prednisolone therapy to complete remission (CR) or partial remission (PR) or maintenance therapy afterwards. As control group we have used peripheral blood samples of the healthy individuals. Undesaturated total mononuclear proteins, using for the Western blotting, were isolated from the all peripheral blood samples, both pts and control.

Results: Immunochemistical method APAAP with using of commercial antibody CPP 32 at the samples of isolated mononuclear cells from peripheral blood all pts have detected presence of protein product gene of caspasa 3. The results of Western blotting analysis have shown that level of activated form of caspasa 3 expression is significantly higher in the peripheral blood samples of CLL pts comparing with the control group.

Conclusion: The results of our pilot study shows that activation of the caspasa 3 protein happens during the execution phase of apoptosis of B-CLL cells. In the samples of peripheral blood total proteins in all the examined pts, by using Western blotting, increased level of activated form of enzyme was detected comparing to protein quantity. Our preliminary results might be valuable predictor of achieved therapy in correlation with the stage of disease and other prognostic factors.

DRUG-INDUCED APOPTOSIS IS MEDITATED BY CONFORMATIONAL CHANGES OF BAX AND BAK IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: Bak and Bax are two members of the Bcl-2 family involved in the regulation of apoptosis. The role of these proteins in the apoptosis induced by several drugs in B-cell chronic lymphocytic leukemia (CLL) cells has been analyzed.

Methods: Cells from 33 CLL patients were incubated for 24 hours with fludarabine (5 μM), dexamethasone (10 μM) or the combination of fludarabine (1 μM) with mafosfamide (1 μM), the active form of cyclophosphamide in vitro, and mitoxantrone (0.5 μM/L) (FCM). Cell viability was determined by Annex V binding. Conformational changes of Bax and Bak were studied using antibodies that recognize the amino terminal of these proteins, a region only exposed during apoptosis induction. Antibody binding was analyzed by flow cytometry and fluorescence microscopy. Cellular distribution of the proteins was analyzed by Western Blot.

Results: A strong correlation was found between the number of apoptotic cells and the percentage of cells stained with antibodies recognizing conformational changes of Bax (n=33, r=0.836, p<0.001) or Bak (n=10, r=0.948, p<0.001). Preincubation of CLL cells with 2-VAU, a broad caspase inhibitor, abolished caspase-3 activation, exposure of phosphatidylinerse residues, and reactive oxygen species (ROS) generation, partially reverted the loss of transmembrane mitochondrial potential (ΔΨm), but did not affect Bax or Bak conformational changes. These results indicate that the conformational changes of Bax and Bak occur upstream of caspase activation or are caspase-independent. Following drug-induced apoptosis, Bax integrates into mitochondria, as demonstrated by fluorescence microscopy and Western blot, without changes in the total amount of Bax or Bak protein. p53 stabilization induced by fludarabine and FCM may not be essential in inducing Bak and Bak conformational changes, because they are also observed in dexamethasone-treated CLL cells.

Conclusions: These results demonstrate that, in CLL cells, the change in the intracellular localization of Bax from cytosol to mitochondria, and the conformational changes of Bax and Bak are one of the early steps in the induction of cell death.

CHEMOKINES AND CHEMOKINE RECEPTORS INVOLVEMENT IN MULTIPLE MYELOMA

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Introduction: Chemokines and their receptors have been identified as pivotal molecules which regulate the trafficking of B lymphocytes. Although several information is provided on normal B lymphocytes and on malignant B cells obtained from patients with non-Hodgkin’s lymphomas, no data are nowadays available on plasma cells obtained from patients with multiple myeloma.

Methods: In this study freshly isolated malignant plasma cells isolated from 20 patients with multiple myeloma, 3 myeloma cell lines and 5 normal PC suspensions were investigated by flow cytometry for the expression of chemokine receptors, including CCR1, CCR2, CCR3, CCR5, CXCR4, CXCR2, CXCR3, CXCR4, CXCR3, and for the ability of several binding chemokines to induce migration, i.e. BCA-1, SDF-1α, SDF-1β, MIP-1α, MIP-3β, Eotaxin-2 and RANTES.

Results: Flow cytometry analysis showed that CXCR4 is regularly expressed on PC obtained from MM patients and MM cell lines. Other receptors, i.e. CCR1, CCR5, CXC4, CXCR2, CXCR3 and CXCR3 are commonly absent on myeloma cells obtained from patients, while CCR1 and CXCR3 was expressed in RPMI-8226 and OPM-2 cell lines. Concerning other receptors (CCR2, CCR3 and CCR6) the expression was variable in patients while they were usually absent on cell lines that have been tested. The analysis of migratory capacity of plasma cells was performed using Boyden chamber in the presence or absence of chemokines (SDF1α, SDF1β, BCA-1/BLC, Eotaxin, RANTES, MIP1α, MIP3α, MIP3β) in some patients a consistent migration was observed in the absence of any exogenous chemotactic stimulus. The migratory response to chemokines used was related to the pattern of expression of chemokine receptors.

Conclusions: These observations indicate that myeloma cells have variable capability to migrate in vitro, this property being more evident in patients with progressive disease or in subjects with the leukemic form of the disease.
LH AND RS CELLS OF HODGKIN’S LYMPHOMA OVEREXPRESS HSP70 AND HETEROGENEOUSLY CASPASE 3.

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Introduction: The purpose of our study was to evaluate immunohistochemical expression of HSP70 and Caspase 3 in selected cases of Hodgkin’s disease, since HSP70 seems to have antiprototic activity while caspase 3 belongs to the effector cascade leading to apoptosis.

Methods: Serial histological sections of 3 cases of N-LPHD, 2 cases of lymphocyte rich C-HD, 13 cases of nodular sclerosis c-HD were immunostained for HSP70 and caspase 3. AEC and DABAB were used as chromogens after secondary biotinated or polymer-linked antibody.

Results: The diagnosis of the various forms of HD was based not only on hematoxylin-eosin stain but also on immunohistochemistry with the use of an ample panel of antibodies. LH cells and RS cells all overexpressed HSP70 while they were heterogenous for caspase 3 expression.

Conclusion: This was a preliminary work on the immunohistochemical phenotyping of HD for HSP70 and caspase 3. Our result are worth further investigation.