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LYSIS OF BONE MARROW STROMAL MICROENVIRONMENT BY POLYAMINE SECRETING EBV-B-CELLS AND PRODUCTION OF INFECTIOUS VIRIONS CAN BE INHIBITED BY THE POLYAMINE SYNTHESIS INHIBITOR MGBG (METHYLGLYOXYLISBIGUANYLHYDRAZONE)

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Malignant hematologic diseases such as lymphomas and leukemias are associated with elevated levels of polyamines (putrescine and spermidine). As the worth of polyamines as biochemical markers for some malignancies has been repeatedly evaluated, they can also be considered as a target for therapy. The model system for this study is a novel type of EBV positive B-cell lines (SSB lines) derived from lymphoma patients. These lines secrete elevated levels of spermidine and spermine which induce lysis of the bone marrow stromal cell layer from which they arose. We performed specific in vitro studies for defining the effect of the polyamine synthesis inhibitor MGBG (methylglyoxalbisguanylylhydrazone) in the SSB line model. It could be clearly shown that MGBG can readily and strongly antagonize proliferation of transformed cells. It has also a protective effect for BM stroma by inhibiting PA mediated lysis. Furthermore it could be shown that MGBG can prevent the production of infectious EBV particles. So it can be concluded that SSB lines provide an ideal model for studying the stroma protective properties of MGBG.

PRESENCE OF T-LYMHPOTROPIC RETROVIRAL (STLV-1) GENOM IN LYMPHOMA CELL DNA AND MORPHOIMMUNOLOGICAL PHENOTYPE OF T- CELL LYMPHOMAS OF VIRUSPOSITIVE BABAONS.

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Some genomic differences between STLV-1 of lymphomatous (STLV-1L) and healthy (STLV-1N) baboons have been revealed formerly. Data presented here show that STLV-1 provirus was detected in all seven cases of investigated baboon T-cell non Hodgkin's malignant lymphomas of different type: lymphocytic and immunoblastic with different immunological phenotypes - helper/suppressor (CD4+CD8+), mixed cell (CD4+CD8+) and double positive (CD4+CD8+). PCR and sequence-analyses demonstrated in all cases presence of STLV-1 with the level of STLV-1L homology to HTLV-1 in 82.4%, 95.9% and 94.9%, and 85.5% for env and tax genome fragments, respectively. STLV-1 detected in peripheral blood lymphocytes and lymph node cells of healthy viruspositive baboons belonged to STLV-1N.

Comparative analysis of DNA env-fragments of M.arctoides STLV-1 provirus (STLV-1ma) obtained from macaque lymphoma and from rabbit lymphoma induced by inoculation of STLV-1ma producing cell line.

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Experimental malignant lymphomas were produced on monkey (M.arctoides) earlier. Parenteral inoculation of rabbits with STLV-1ma producing lymphoid cell cultures (MAL-1), exhibited oncogenic activity. Incubation period was short and lasted for several weeks, lymphomas were fatal and generalized. Using PCR method and primers to conservative env-fragments of HTLV-1/STLV-1 sequences of STLV-1ma env-gene have been detected in M.arctoides lymphoma DNA, in cell culture MAL-1, and in DNA of induced rabbit lymphoma. Amplified by PCR fragments of env genomic of STLV-1ma from M.arctoides, cell lines and rabbit lymphoma were sequenced. Comparative sequence analysis showed that most of nucleotide substitutions on sequenced fragments (81.8%) were translation- silent point mutations of transition type. Computer hydrophobic analyses and analyses of predicted secondary structures of immunodominant part of ENV protein STLV-1ma showed high conservation of these fragments which is characteristic of other representatives of retroviruses of HTLV-1/STLV-1 subfamily.

Expression of Homeobox B genes in malignant lymphoma

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Homeobox (HOX) genes may have a regulatory function in differentiation process in hematopoiesis and may participate leukemogenesis. The previous reports showed that the expression of HOX B cluster was largely restricted to erythroid cell line. No HOX B gene is expressed in the quiescent lymphocytes but expression of selected HOX B genes were shown in a majority of acute lymphoblastic leukemia. In the present study, we examined the expression pattern of HOX B genes in malignant lymphoma and whether aberrant expression of HOX B genes is responsible to lymphomagenesis. Twenty-one lymph node samples of non-Hodgkin's lymphoma (7 T-NHL and 14 B-NHL), four lymph node samples of Hodgkin's disease (2 M and 2 NS) and 5 lymph node of reactive lymphadenitis were studied. Total RNA was extracted by the AGPC method from the freshly biopsied lymph node and the expression of HOX B, B8 and B9 mRNA was examined by RT-PCR method. T-NHL expressed mRNA of HOX B6 (6/6), HOX B8 (3/7) and HOX B9 (5/7) and B-NHL expressed mRNA of HOX B6 (5/4), HOX B8 (6/7) and HOX B9 (6/7). Hodgkin's disease expressed mRNA of HOX B6 (3/4) and HOX B9 (2/4). No HOX B genes were detected in the lymphadenitis and normal lymphocytes. The expression of HOX B6 and B9 mRNA was shown more frequently in T-NHL and NS of Hodgkin's disease than in B-NHL and MC, respectively. HOX B gene was expressed specifically in T-NHL, in malignant lymphoma. These results suggest that the expression of some HOX B genes in malignant lymphoma may represent a significant oncogenic cofactor.
STSA/(ST) and MSM/Ms(MSM) mice are extremely resistant to lymphomagenesis by the exposure to radiation, while BALB/c (BALB/c) mice are highly susceptible. We analyzed the loss of heterozygosity (LOH) at polymorphic microsatellite loci and cytogenetic status in radiation-induced lymphomas of (BALB/c × STS)F1 and (BALB/c × MSM)F1 hybrid mice. Highly frequent LOHs were observed in the regions on chromosomes 4, 12, and 19 of the lymphomas of (BALB/c × STS)F1 hybrid mice and in the region on chromosome 12 of (BALB/c × MSM)F1 mice, although the frequencies of LOH were low in other regions examined so far. Loci located at 57 cM from centromere on chromosome 12 were lost the most frequently in both hybrid mice. In only chromosome, a loss of alleles derived from STS mice, a resistant strain to radiation lymphomagenesis, was significantly more frequent than that of BALB/c mice. Cytogenetical analysis of (BALB/c × STS)F1 lymphomas with LOHs both on chromosome 4 and on chromosome 12 or 19 showed no obvious chromosomal deletion in the regions which the LOHs were observed. These results suggest the generation of partial uniparental disomy probably by nondisjunction and/or mitotic recombination. The generation of such uniparental disomy may play important roles in lymphomagenesis by radiation which induces DNA strand breaks and rejoining.

**FUNCTIONAL DIFFERENCES BETWEEN MATURE AND PRIMITIVE PLASMA CELLS**

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Malignant plasma cells from patients with myeloma display considerable heterogeneity of immunophenotype. Plasma cells express high intensity CD38 (CD38+), cytoplasmic immunoglobulin (IgG+), and either kappa or lambda light chains. Subpopulations of mature CD38+/CD45+, CD45+, CD19-, immature CD38++, CD45+ and primitive CD38++, CD45+ and CD19+ plasma cells can be defined but little is known about the functional differences and the clinical significance of these subpopulations. Three colour flow cytometry was used to determine the labelling index (chromoconcentration), number of cell membrane nucleoside transporters per cell, p-glycoprotein (intracellular JLB), IL-6 receptors and oncogene protein (c-myc, c-fos, c-neu, bcl-2, p53, p57, and PKC) of plasma cell subpopulations. The mean number of plasma cells in each subpopulation from the bone marrow of 65 patients was mature (55%), immature (29%) and primitive (12%). When compared with mature plasma cells, primitive plasma cells had a significantly higher labelling index (mean = 7.0% vs 1.8%; t = 3.8, p < 0.001), a higher expression of c-myc protein (55.3% vs 5.6%; t = 9.6, p < 0.001), p-glycoprotein (12.2% vs 3.4%; t = 2.3, p < 0.05) and number of nucleoside transporters per cell (5554 ± 2219). Nineteen percent of patients had > 10,000 transporters per cell on their CD38++ CD45+ cell population. There was a significant difference in the mean labelling index of the CD38++ CD45+ population in patients with progressive compared to stable disease (9.2% vs 2.2%; z = 19.9, p < 0.001). Labelling indices of primitive plasma cells ranged up to 46% and provided a better correlation with disease status (p < 0.001) than the total plasma cell population (p < 0.01). The rising Li seen in escape disease is due to a rise in the Li of the primitive plasma cell population with little or no change in the Li of the mature plasma cells. Relative proportions of primitive and mature plasma cells do not change with disease escape, therefore there exists a highly proliferative pool of primitive plasma cells which are the cause of progressive disease.
Does impaired cytokinesis of Hodgkin-Reed-Sternberg (HRS) cells correlate with monoclonality of Hodgkin's disease?


Impaired cytokinesis of HRS cells has been shown both in vitro and in vivo, where most CD30+ cells exhibit abortive mitoses, with a highly significant arrest at the metaphase-ana(tele)phase transition. The clonality of HRS cells have been investigated in different ways, the development of PCR offers a new and powerful approach to the study of HD. Few studies show the presence of little gene rearrangement in HRS cells from some cases of HD, whereas other studies demonstrate polyclonal IgH gene rearrangement. In this study we have addressed the question whether different kinetic characteristics of HRS cells in HD cases correlate with the detection of monoclonality.

54 cases of HD (NS3;MC16) have been analyzed for light gene rearrangement using FR3A and FRD3A primers. Cell counts were carried out in sections immunostained for CD30 and, in each case, we have registered (1) the mean number of CD30+ and CD30- lymphoid cells per high power field; (2) the percentage of mononuclear and multinuclear CD30+ cells; (3) the Mean Index (MI) and (4) the fraction of CD30+ mononuclear cells in an identifiable phase pro-meta-ana or ana(tele)phase. In addition, the Endomitotic Index (EI) and the Proliferative index (PI) was calculated where EI = number of nuclei in multinucleated CD30+ cells/100total number of nuclei of CD30+ cells and PI = MIA/9(MIA+Tel5/100).

An light rearrangement was demonstrable in 14 cases (NS 5; MC 6,8) out of 54 classical HD. These monoclonal cases are characterized by HRS cells with significantly higher MI, more numerous ana(tele) phase figures, less frequent multinucleation and endomitosis than a group of cases in which monoclonality is not shown. In such cases, another consistent sign of non-monoclonal (by PCR) cases have HRS cells with the same morphologic characteristics and proliferation pattern of monoclonal cases. In monoclonal and in monoclonal-bearing cases the mean number of CD30+ lymphoid cells is significantly lower than in the other cases, however, the mean number of CD30- cells is not different.

Monoclonality is more frequently associated with the capacity of CD30+ cells to complete the mitotic cycle and with the depletion of CD30- lymphoid cells, so giving a more uniform, neoplastic likelihood to the cell population.

Differential expression of A- and B-type lamin in cells of Hodgkin's disease as compared to reactive lymph nodes

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Lamins are nuclear membrane associated proteins. In general, it is assumed that lamina A and C are preferentially expressed in differentiated cells, while proliferative cells do not display these lamina. Lamin B1 and B2 are thought to be exhibited constitutively in all cell types. Hodgkin's disease and its normal counterpart, the reactive lymph node were investigated immunocytochemically with A- and B-type specific monoclonal antibodies to study the degree of differentiation and posttranscriptional regulation in the different cell types present in these tissues.

Analysis of the different cell types in both Hodgkin's disease and the reactive lymph nodes showed a heterogeneity in lamin positivity. The highly proliferative B lymphocytes of the follicle centre in the reactive lymph nodes expressed only lamin B1, whereas these cells stained not or only very weakly with lamin B2, A and A/C antibodies. Mantle zone lymphocytes displayed lamin B1 and B2 but were completely negative for A-type lamin antibodies. Furthermore, all CD30 positive and CD20 positive lymphocytes in the medulla and paracortex lacked A-type lamina. In Hodgkin's disease, all cells had lamina B1 and B2, whereas A-type laminae were primarily observed in Reed-Sternberg and Hodgkin cells.

Our findings indicate that A-type laminae are not displayed in B and T lymphocytes of the reactive lymph nodes, whereas this type of lamina is principally observed in the Reed-Sternberg and Hodgkin cells of Hodgkin's disease. This suggests that these malignant cells are more differentiated than the surrounding lymphocytes. Finally, our results on the lack of lamin B2 expression in the follicle centre indicate that B-subtype laminae are not constitutively expressed in all nucleated cells.

Genotypic analysis of the Hodgkin's tissue derived cell line HKB-1

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Objective: Neither lineage specificity nor differentiation of tumor cells in Hodgkin's disease (HD) are clear. In some cases of HD, the npm/alk translocation was found. In this study, genotypic analysis of a novel Hodgkin's tissue derived cell line, HKB-1, is presented.

Background: Phenotypically the cell line HKB-1 is positive for B cell markers (CD19, CD20, CD23) and for the HD-associated antigens CD30 and CD15, as previously reported. Epstein-Barr virus (EBV) infection of HKB-1 was excluded by failure to detect EBV DNA and EBV related antigens.

Methods: Repeated karyotyping was performed by standard cytogenetic methods. DNA of HKB-1 was analyzed for immunoglobulin heavy-chain (Igh) gene rearrangements and for T cell receptor (TCR) gene rearrangements. In order to examine the cell line for the translocation t(8;14) (p23;q32), RNA was investigated by a RT-PCR assay in addition to immunohistotyping using an 8A specific antisera (anti-8A).

Results: Cytogenetic analysis showed a stable pseudodiploid karyotype with complex clonal aberrations. Although structural aberrations included band 2p23, no fusion product representing the npm/alk translocation was found neither on protein nor on RNA level. HKB-1 cells harbour clonal light chain rearrangements whereas no TCR gene rearrangements were detected.

Conclusion: The clonal light chain rearrangements confirm the B cell origin of HKB-1 and may indicate that the cell line is of tumor origin. For HKB-1, (2;15) seems not to be involved in cellular proliferation.

Immuno- and Gene Therapy of Hodgkin's Disease (HD): A Hodgkin and Reed Sternberg (HRS) Cell Specific Restin Transcript as a Target.

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Using autologous serum for the serological analysis of recombinantly expressed clones (SEREX) from a cDNA library derived from a spleen involved by HD, several new HD associated antigens were identified which are immunogenic in the autologous host. Sequence analysis demonstrated that one of these antigens, HGM-HRS-397, is encoded by the gene for restin, an interaline filament associated protein, which has recently been described to be specifically expressed in Hodgkin and Reed Sternberg (HRS) cells. The comparison of the identified transcript with the published restin sequence revealed that the cDNA codes for a protein which is truncated at the carboxy terminus leading to the disruption of the α-helical rod domain, which is of paramount importance for the formation of filaments. Northern blot analysis demonstrated specific expression of the transcript in tissues involved by HD, but not in normal tissues. Expression of this new restin molecule was demonstrated in HRS cells by single cell RT-PCR with oligonucleotides specific for the identified transcript. Western blot analysis with polyclonal rabbit serum produced by immunization with recombinantly expressed antigen confirmed the expression of a truncated protein in HD involved tissue. Further analysis will explore the role of this molecule in the pathogenesis of HD. Because of their immunogenicity in HD patients and their suggestive pathogenetic role, HRS specific restin and other HRS antigens identified by SEREX are prime candidates for immuno- and gene therapy approaches in HD.
COMPARATIVE ANALYSIS OF GENE EXPRESSION IN HODGKIN’S DISEASE DERIVED AND LYMPHOBLASTOID CELL LINES BY DIFFERENTIAL DISPLAY

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Molecular studies (Küppers-R et al., PNAS (1994) 91, 10902-10906) provide evidence for a B-cell like nature of Hodgkin (HD) - Reed-Sternberg cells. Against this background we compared the mRNA expression pattern of a panel of HD derived cell lines (Li236, Li540, Li428) and human lymphoblastoid cell lines (EBV transformed B-cells) by means of the differential display method (Liang-P. Pardee-AB, Science (1992) 257, 967-71). Signals restricted to the panel of HD cell lines were assigned as HD-related and the respective cDNAs were cloned via a commercial T/A vector system (Invitrogen) and sequenced. Sequence data were compared with EMBL-databases (EST, virus and primate division) using the FASTA algorithm to identify homologies. Candidate clones representing HD restricted gene expression are now validated by a ribonuclease protection assay utilizing mRNA purified from a variety of HD derived cell lines. The majority of sequences obtained so far have no match in the accessible databases and thus may represent novel transcripts restricted to HD. Sequence data will be submitted to public-domain EST databases after unequivocal validation of the HD restricted expression pattern. The differential display method proved to be suitable for the rapid screening of differentially expressed genes in Hodgkin’s disease derived cell lines.

DIFFERENT MOLECULAR CONSEQUENCES OF A t(9;22)(q34;q11) IN CML AND NHL

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The t(9;22)(q34;q11) resulting in the BCR-ABL gene fusion is a classical chromosomal translocation observed in almost all CML cases and in 5% of patients with NHL. Although cytogenic for CML, a t(9;22) has been found in more than 10 lymphoma patients. We report here an unusual case of follicular lymphoma without evidence of histological progression towards a high grade lymphoma carrying simultaneously a t(9;22)(q34;q11), t(14;18)(q32;q21) and t(8;14)(q24;q32). The latter two translocations were confirmed by molecular studies which showed also rearrangements of Ig heavy, kappa and lambda loci. Investigation of the t(9;22) using Southern blot and RT-PCR analysis did not detect MCR or MBR rearrangements of BCR, typically associated with a t(9;22). Moreover, two colour FISH with ABL and BCR probes confirmed a t(9;22), but the "fusion" signal was not found on a der(22) like in CML, but on a der(9) suggesting that both these translocations are different on a molecular level. Further FISH analysis using 9q34 and 22q11 specific probes identified a breakpoint on chromosome 9 distally to the CAN gene located 350 kb downstream of ABL and a breakpoint on 22q11 telomic to the DI George locus, thus in a region carrying the Ig lambda gene cluster. Interestingly, FISH analysis of other 3 NHLs with various 9q34 abnormalities revealed that in all these cases the 9q breakpoint occurred at the telomeric side of CAN, like in the reported t(9;22). Whether the same target gene at 9q34 is deregulated in all these lymphoma cases remains to be determined.

In conclusion, t(9;22) found in an NHL patient, cytogenetically indistinguishable from a t(9;22) in CML, appeared to be molecularly distinct and apparently does not involve ABL and BCR genes which are rearranged in Ph+ CML/ALL cases.
Aggressive NK non-Hodgkin's Lymphoma with a t(X;18)(q13;p11).


We report the case of a 24 year old female patient admitted to our institution for pain, fever, right pleural and pericardic effusions, mesenteric lymph nodes and ulcerated infiltrating colic lesion. Biopsy specimen identified a malignant lymphoma with histologic characteristics of an angiocentric growth pattern. Despite an intensive chemotherapy regimen, the patient died 1.5 month later.

Cytologic analysis (pleural effusion, lymph node and tumor implants) showed that most of the neoplastic cells contained numerous and voluminous azurophilic granules. Immunophenotype was in accordance with a NK origin since cells were positive for CD2, CD7, CD8, and CD56 and were negative for CD3, CD5, CD19, CD20, and Igs. No clonal rearrangement of the immunoglobulin heavy chain gene or the T cell receptor loc (TCR b, y, d) was detected. In situ hybridization studies revealed the presence of EBER RNA in many tumoral cells. Cyogenetic analysis performed on pleural cells found an abnormal clonal caryotype, 45, X,-X, der(18)(X;18)(q13;p11), confirmed by the double color FISH assay with the two appropriate centromeric probes. Recurrent chromosomal abnormalities have never been described in NK-derived NHL not only because of the infertility of this hemopathy but also because of the difficulty to obtain abnormal karyotype. Der(18)(X;18) can be therefore considered as a new primary non-random translocation of NK NHL.

BCL-6 gene product, a 92- to 98-kDa nuclear phosphoprotein, is highly expressed in germinal center B-cells and their neoplastic counterparts

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The BCL6 gene is known to be located on chromosome 3q27, at the breakpoint of the 3q27-associated translocations that occur frequently in human non-Hodgkin's lymphomas (NHL). In order to identify the BCL6 protein, two antibodies that recognized distinct domains of this protein were raised in rabbits. Immunoprecipitation and immunoblotting of lysates of BCL6-expressing cells using both antibodies demonstrated a broad 92-98 kDa band. De phosphorylation of BCL6 protein reduced the size of this band to 87 kDa, suggesting that BCL6 may be expressed in a phosphorylated form. Immunostaining with both antibodies revealed that BCL6 protein was localized in the nuclei of most of the germinal center B-cells and a small number of marginal zone B-cells. Furthermore, BCL6 protein was expressed in follicular, Burkitt's, and diffuse large B-cell lymphomas. These results suggest that the BCL6 protein, expressed in B-cells of the germinal centers which are important in the maturation of immune responses, may play some physiological role(s) in the germinal center B-cells.

IgH ALTERATIONS IN PROGRESSIVE LYMPHOMAS


Introduction: Alterations in clinical behaviour, morphological appearance and immunophenotype are recurrently observed in relapsing non-Hodgkin's lymphomas (NHL). The aim of this study was to analyse a panel of relapsing or progressive lymphomas with respect to clonality changes, using the immunoglobulin heavy-chain (IgH) as a marker of clonality.

Material and methods: 72 samples taken during the course from 19 NHL cases were investigated with a specific analysis of IgH locus. VH gene family-specific PCR-SSCP and in five selected cases by reverse analysis of VH gene fragments. Seven cases showed transformation or de novo lymphomas during the course.

Results: In 10 cases no alteration of the IgH locus could be detected by the methods used. Five BFLP S/7 cases with transformed/deciduous lymphomas and 3/12 with unaltered morphology showed altered IgH pattern. Altered VH gene PCR-SSCP pattern and/or VH gene family utilization was observed in 7 cases, 6 of these also showing altered IFLP. In one case, a rearrangement involving an additional VH family was amplified at relapse. In two other cases the VH rearrangement from diagnosis was not detected at relapse. Sequence analysis revealed point-mutations in the 3 cases (A,G,C,D, respectively) with altered PCR-SSCP pattern. In the case with a novel VH gene family utilization at relapse, subclones with two different VH gene replacements were detected. In one case, different subclones in different compartments were observed.

Conclusion: Alterations of the clonal IgH rearrangements during the course occurred in about half of the cases, mainly in the transformed/deciduous lymphomas and were due to point mutations as well as VH gene replacements in the malignant cells.
COEXPRESSION OF THE TUMOR NECROSIS FACTOR (TNF)/LYMPHOTOXIN (LT)
AND FAS LIGAND-RECEPTOR mRNA IN NON-HODGKIN LYMPHOMAS: HOST IMMUNE RESPONSE AGAINST NEOPLASTIC B CELLS

2. Biology
Detection of Antigen Receptor Gene Rearrangements by SSCP Analysis

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Although antigen receptor (AGR) gene rearrangements are not involved in the generation of lymphoid neoplasms, they are frequently used to detect malignant lymphoid clones. PCR procedures applying consensus primers for framework sequences generate amplifications of equal sizes but differing in their sequences. For the distinction between different rearrangements cloning and sequencing steps are required which ate time consuming and experimentally demanding. Therefore single-strand conformation polymorphism (SSCP) analysis was assessed for its potential to distinguish between the different amplificates. Variable AGR gene rearrangements were amplified from plasmid templates containing cloned VDJ regions as well as from genomic DNA extracted from B-cell lines or blood samples from lymphoma patients or healthy donors. Using either consensus primers specific for regular IGH-rearrangements or primers for IgH-bc12 fusions resulting from I(14;18) translocations, PCR products of identical size but with a high degree of sequence variation were obtained. Convenient and reliable differentiation of such amplificates by SSCP analysis was achieved by a standardized denaturation procedure prior to separation in polyacrylamide gels, that were subsequently silver stained. Serial dilutions of a patient's genomic DNA in a healthy donor's genomic DNA showed that idiotype specific sequences can still be detected at a more than 1000-fold excess of polyclonal rearrangements. Thus SSCP provides a rapid and easy method for monitoring clinical disease which can be routinely performed during the treatment of lymphoma and leukaemia patients.

NON HODGKIN'S LYMPHOMAS (NHL): COMPARISON OF Mab Mbi1, K67 AND NUCLEAR ORGANIZER REGIONS (NORs)

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The monoclonal antibodies Mbi1 and K67 recognize antigenic present in the nucleus of proliferating cells. We examined 23 lymph nodes, half of these paraffin embedded and half snap-frozen in liquid nitrogen. The purpose of this study was to investigate the relationship between Mbi1 performed in paraffin sections and K67 performed cryostat sections. Paraffin sections were cut at 3 μm and immunostained with Mab Mbi1 (Immunotech), secondary antibodies were revealed with peroxidase-antiperoxidase complex (LSAB KIt, DAKO) using DAB substrate (Biogenex, California USA). Cryostat sections were cut at 4-5 μm and immunostained with K67 (Dakoopatik UK Ltd) using an APAAP kit method (Dakoopatik UK Ltd). Two of us, individually, scored the Mbi1 sections and the other two scored the K67 sections. We used 3 grade of positivity: 1 = 0-29% positivity; 2 = 30-70% positivity; 3 = more than 70% positivity. The results showed that 82.65% of cases presented complete correspondence between Mbi1 and K67 section and four cases were minimally discordant. Eleven cryostat sections were also stained with the silver nuclear organizer regions (AgNORs) staining solution for 9 minutes at 37°C in the dark. We examined the silver stained sections without knowledge of histological diagnosis or the results of the two monoclonal antibodies. The number of NORs in a minimum of 100 cells was counted and a mean score determined for each case. We observed that if we divided the score of AgNORs in 0-2, 3-5 and more than 6 black dots. We have good correspondence with K67 (77 correspondence in 10-2 in group 0-2, 2/2 in group 3-5 and 2/2 in group >6) and a lower correspondence with Mbi1 (6/7 in group 0-2, 3/7 in group 3-5 and 2/2 in group >6).

We can conclude that the high correspondence between the two monoclonal antibodies suggest that they could recognize a common antigen. The AgNORs technique can be utilized as rapid solution to demonstrate cellular kinetics of lymphoma.

COMPARISON OF PCR AND SOUTHERN ANALYSIS IN THE DETECTION OF CLONAL CELLS CARRYING THE BCL2/IgH TRANSLOCATION IN BONE MARROW OF PATIENTS WITH NON HODGKIN LYMPHOMA

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The bcl2 translocation into the JH region of the immunglobulin heavy chain gene is widely accepted as a hallmark of follicular lymphoma. We investigated 14(18) (q32;q21) within the major breakpoint region in bone marrow of 35 patients. 25 of 35 patients were positive in PCR while 11 of 18 showed comigrating rearranged bands in Southern Analysis. Of the patients with histologically verified bone marrow infiltration, 11 of 16 were positive in PCR compared to 8 of 9 positive in Southern Analysis. Of the patients with negative bone marrow histology, 11 of 16 were positive in PCR compared to 3 of 9 positive in Southern Analysis. Thus PCR detects bone marrow infiltration in a considerable number of cases with negative bone marrow histology but Southern Analysis has a higher detection rate in cases with positive bone marrow histology. Our results provide further evidence that the sensitivity of both methods is limited and that they should be combined for monitoring patients with follicular Non Hodgkin lymphoma.

EFFECTS OF ANTIENGLOSTOMIC CHEMOTHERAPY ON IMMUNOLOGICAL AND VIRAL PARAMETERS IN PATIENTS WITH NON-HODGKIN LYMHPHOMA AND HIV INFECTION


Objective: The aim of this study was to estimate the efficiency of chemotherapy (CT) on immunological and virological parameters in patients (pts) with non-Hodgkin lymphoma (NHL) and HIV infection. Methods: Five pts with documented history of seroconversion for HIV-1 antibody and with diagnosis of NHL were treated with CHOP regimen (Cytosine-Cytosine-Folinic Acids (CHOP) and intravenous PEGIFN-alfa 2a, four median cycles were scheduled every 21 days. At the end of the regimen, 3 cycles of therapy and after 1 month following the end of treatment, the absolute number and percentage of the CD4+ and CD8+ lymphocytes were evaluated by flow cytometry. Moreover, plasma samples of patients were estimated by using a p24 ELISA and a RT-PCR competitive assay, before the end of therapy and after 1 month from the end of chemotherapy. Immunological data were analyzed with the student's t-test and were considered statistically different when p < 0.05. Results: Baseline characteristics of pts: median age 42 years (range; 35-55); 1 intravenous drug user, 2 homosexual sex; 1 heterosexual sex; the clinical staging for HIV infection according to 1997 CDC classification, before the NHL diagnosis was: 2 II, 2 IVD and I VI; 4 pts were previously treated with antiretroviral compounds. With regard to the biological subtypes of NHL, 2 pts had intermediate grade lymphoma (G3) according to Working Formulation (WF) and the other three had high grade lymphomas (G4, 1 II, 1 II according to WF). All pts received 100% of CHOP plus G-CSF and they followed regularly the schedule of treatment. Before the beginning of CT the mean value of CD4+ percentage was 21 ±1.8% and there was a significant decline (p = 0.01) after 1 month from the end of treatment (7 ± 4%). While the mean value of CD4+ increase at the initiation of CT 21±11%, it decreases significantly already after the third cycle of CT (19±3.9%, p = 0.04) and it remains significantly low after 1 month from the conclusion of CT (18±2%, p = 0.03). Double therapy, the mean values of CD4+ percentage and CD4+immunoglobin remain stable and during the therapy. There is a significant variation of serum p24 antigen levels, but plasmatic viremia, measured with a more sensitive quantitative assay, showed a significant increase in the number of viral RNA molecules (0.35-1.5 log10) between the second and third CT cycle; then it descends to the initial base line values. Conclusions: Our results show that CHOP regimen in HIV pts causes a selective decrease of CD4+ lymphocytes and only a transient increase of viremia. On the other hand, literature documents a decline in both CD4+ and CD8+ lymphocytes during CHOP and CHOP-like CT in pts with HIV and without HIV infection. Further investigations are undergoing.

2. Biology

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NON-HODGKIN'S LYMPHOMA IS NOT RELATED TO HEPATITIS-C VIRUS INFECTION.


Hepatitis-C virus (HCV) infection has been associated with certain subgroups of non-Hodgkin's lymphoma (NHL) with seroprevalence up to 30%. We have analysed seroprevalence of HCV infection in all 115 unselected patients with NHL who visited our outpatient clinic of Hematology during a period of 6 weeks. The study included 69 men and 46 women with a mean age of 53 ± 15 yrs, range 19-84 yrs. The NHL were classified according to the Kiel classification and grade of malignancy was assigned according to the International Working Formulation. Twenty-seven patients (23%) were previously untreated. All others were either receiving chemotherapy or were in remission after prior treatment. Anti-HCV were detected by second-generation enzyme immunoassay (IBase HCV, ELISA; Abbott). In case of a positive result, an additional confirmatory test was performed (RIBA; Chiron Corp. Emeryville, CA).

There were 51 (44%) low-grade, 42 (37%) intermediate-grade and 22 (19%) high-grade NHL. B-cell origin was demonstrated in 99 (86%) cases. All but one patient were tested seronegative for HCV with ELISA. The only patient with positive ELISA proved negative at RIBA confirmatory test. Only eight patients had received prior interferon-alpha (IFN-α). Thus IFN-α treatment could not be held responsible for seronegativity in the vast majority of the patients. Only nine patients had low leukocyte counts <3.5x10⁹/l. Twelve patients had elevated ALAT levels without evidence for viral hepatitis. Previous exposure to blood products was recorded in 31 patients but in only five patients this had occurred prior to 1989.

Our data do not support a relationship between HCV infection and NHL. Possibly, a higher yield of seropositivity could be reached with PCR analysis of virions and will be done in this study group. However, it seems unlikely that the results will change significantly.

SPONTANEOUS AND RADIATION-INDUCED APOPTOSIS IN NON-HODGKIN LYMPHOMAS

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Patients and methods: tumor cells were obtained by lymph node fine-needle aspiration in 49 patients (29 females, 20 males) with either low-grade (21 patients), transformed low-grade (10 patients) or high-grade (18 patients) lymphoma. Nuclear morphology and chromatin-DNA were analyzed by fluorescence microscopy. Apoptosis index was scored immediately after aspiration (Apopto) and 24 hours after 0 Gy (Riso), 2 Gy (Riso) and 10 Gy (Riso10) delivered in vitro.

Results: apoptosis levels could be measured in 43 patients (technical failure rate 12%). The median (range) percentages of apoptotic cells were: Apopto: 4% (1, 45), Riso: 39% (8, 943); Riso: 70% (25, 100); and Riso10: 81% (35, 97). Spontaneous apoptosis (Apopto and Riso) did not vary with gender or histological type. Transformed low-grade lymphomas were less sensitive to 2Gy (in all patients, p = 0.02) and 10Gy (in males only, p = 0.02) than low or high-grade cells. Nineteen patients (11 low-grade, 4 transformed, 4 high-grade) received palliative irradiation (2x2Gy). Histology was not associated with the response to irradiation. Four male patients had a major response: their Riso and Riso10 were significantly higher than in 5 non-responders (p = 0.03 and 0.03, respectively). Nine out of 10 females were major responders, so that the predictive value or radiation-induced apoptosis could not be investigated in female patients.

Conclusion: spontaneous apoptosis did not correlate with histological type. Transformed low-grade lymphomas were less sensitive to radiation-induced apoptosis assayed in vitro. Our data suggest that in vitro radiation-induced apoptosis may predict the tumor response to low doses of irradiation.

Supported by a grant from the Ligue Nationale Contre le Cancer (Comité de Paris)

IN VIVO PROLIFERATION CHARACTERISTICS OF REACTIVE T-LYMPHOCYTES IN B-CELL NON HODGKIN'S LYMPHOMA (NHL)

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Flow cytometric analysis of cell cycle kinetics in overall cell suspension of tumour samples may produce inaccurate results due to admixture of normal reactive cells. In B-cell NHL, normal reactive T-lymphocytes are numerous and even exceed the number of malignant B-cells. Therefore we have analysed the proliferation characteristics of T-cells after in vivo pulse labelling with the thymidine analogue iododeoxyuridine (IldUrd). Nineteen lymph node samples of B-cell NHL were processed to cell suspensions, positively sorted with CD19 for B-cells and CD3 for T-cells and analysed for cell cycle kinetic parameters. There were six low-grade and 13 intermediate-grade NHL. Prior to sorting the median % of B-cells was 61% (range: 15-93) and of T-cells 32% (range: 5-69) in the overall suspension. After sorting, the B-cell suspension contained a % of 98 % of B-cells (range: 90-100 %) and the T-cell sample 98 % of T-cells (range: 93-100 %). The IldUrd-labelling index (LI) did not differ significantly between B- and T-cells: median 1.2 % (range: 0.5-5.3) and 2.3 % (range: 0.67-2.0 respectively (p = 0.1). The LI of the T-cells correlated significantly with that of the B-cells: the higher the LI of the malignant B-cells, the higher the LI of the T-cells (R = 0.38 with p = 0.04). Correlable results were obtained with the growth fraction of B-cells and of % (3-64) for T-cells. The duration of the cell cycle phases revealed no relevant differences in the duration of the S-phase (Ts) with a median duration of 10 hours (range: 6-17) for B-cells and 11 hours (range: 6-13) for T-cells. The median duration the complete cell cycle was somewhat shorter in T-cells with 61 hours (range: 16-174) as compared to 98 hours (range: 73-250) for B-cells. However, the growth fraction was considerably improved for the T-cells (0.57) compared to B-cells (0.24).

In conclusion, reactive T-cells have a remarkable proliferative capacity positively related to that of the malignant B-cells. It remains to be elucidated to what extent proliferation characteristics of T-cells reflect host immune responses to the development and progression of the NHL.

HEPATITIS C VIRUS IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA


Recent publications mention that hepatitis C virus (HCV) is being found with higher frequency in patients with non Hodgkin's lymphomas than in the general population. Mixed cryoglobulinemia with HCV can evolve to low grade lymphoma. In an article of Blood (1994;84:3047-53) out of 31 patients with mixed cryoglobulinemia 12 were found to have lymphoma. We studied a group of 94 non Hodgkin's lymphoma patients from 2 Oncology Clinics of Caracas 61 (64.9%) were diffuse & 33 (35.1%) follicular. We discarded the patients who had been transfused after the diagnosis in order to limit our study to pre lymphoma HCV transmission. Anti-HCV was checked with a 3rd generation ELISA (HCV Ortho 3.0), AntiHIV, anti-HBC and HTLV-I (Ortho) were investigated in all patients.

Results: Ages ranged from 18 to 83 (mean 51), with a male/ female ratio of 0.7. The incidence of HCV was 794 (7.4%), 4 of the 7 were diffuse lymphomas (1 mixed and 1 large cell) and 3 were follicular (2 small cleaved and 1 mixed). Among the 7, none was positive for AntiHIV & 1 was positive for anti-HBC. In the rest of the 87 cases were positives for AntiHIV & 6 for anti-HBC. One patient was positive for HIV. None carried HTLV-I. The frequency of HCV carriers in the blood bank of our Hospital is 0.8% (4,000 tests), this figure is significantly smaller than the one of our study (p < 0.0001). Our finding of a higher incidence of HCV in non Hodgkin's lymphomas than in the general population seems to confirm a previous study (Ibs J Hiematol 1994;84:3047-52). We believe our cases of diffuse histology than follicular ones, this is consistent in Latin America where the ratio can be up to 4 to 1. The positivity for HCV in lymphoma that we found is smaller than the one reported in Italy, this could be related to a lower prevalence in the population of Venezuela.

Acknowledgment: this study was supported in part by Johnson & Johnson Medical de Venezuela.
Growth modulation of freshly isolated Non-Hodgkin’s malignant B-lymphoma cells induced by IL-2, IL-3, IL-4, IL-6, IL-10, IL-13, Interferon alpha, Interferon gamma, GM-CSF, GM-CSF and all-trans-retinoic-acid.

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We investigated the potential of ten cytokines and all-trans-retinoic acid to modulate the proliferative response in vitro of purified B-nonn Hodgkin’s lymphoma cells of various histological subtypes. 32 malignant lymph nodes were studied. The growth of 29 lymphomas could be induced by one (6 cases) or several (23 cases) cytokines or all-trans retinoic acid (ATRA). Modulators most often implicated were IL2 (56% of cases), IL4 (33% of cases), interferons gamma and alpha (50% and 47% of cases). Hematopoietic growth factors (IL3, GM-CSF, G-CSF) were rarely concerned (9% to 14% of cases). In comparison to spontaneous proliferation, the values of growth stimulation ranged from a 1.1-fold to a 25.8-fold increase, and the values of growth inhibition ranged from 11% to 91%. The profile of cytokine response (stimulation, inhibition or no effect) was extremely variable from case to case. No relationship could be found with the histological subtype. Moreover, each cytokine could display either positive or negative influence on the proliferation depending on each lymphoma case studied. Two notable exceptions were IL2, displaying exclusively a positive effect, and ATRA displaying exclusively a negative effect. Overall, these results may have strong implications for future clinical studies using cytokines in the treatment of lymphomas. Ideally, the pattern of in vitro growth response to cytokines or ATRA should be determined individually before undertaking any cytokine treatment.

IL-10, IL-2, IL-6 ARE COOPERATIVE GROWTH FACTORS FOR B NON HODGKIN’S LYMPHOMA CELLS.

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IL-6, IL-10 and IL-2 are growth factors for human lymphoma cell lines. Increased serum levels of IL-6, IL-10 and IL-2 have been reported in vivo in patients (pts) with NHL. The in vitro production of IL-10, IL-6 and IL-2 in NHL tumor samples was investigated by immunohistochemistry (IHC) in a series of 34 pts (38 high and 16 low grade). Respectively 55%, 89%, 20% and 31% of tumor samples were positive for IL-10, IL-6, IL-2 and TNF by IHC. Using RT PCR, IL-10 and IL-6 mRNAs were detected in 100% of the samples, whereas TNF or IL-2 mRNAs were detected in 90% and 31% of the samples, respectively. In 13 of these 34 pts, tumor tissue was available for B NHL cell purification with Dynal beads. IL-2 and IL-2 receptor were detected neither in supernatant nor by IHC on purified tumor cells. TNF, IL-10 and IL-6 were detected in the supernatants of 31%; 80% and 86% of purified tumor cells. No correlation between serum dosage and IHC detection was observed for IL-2, IL-2 receptor and TNF. Five of 9 patients with detectable serum IL-10 had a detectable IL-10 production by tumor cells. Ten of 12 patients with detectable serum IL-6 had a detectable IL-6 production by tumor cells. The growth promoting effects of IL-10 alone and with IL-2 and IL-6 on fresh NHL B cells (cultured on a feeder of fibroblast L cells transfected with FcRγII/CD82 with an anti-CD40 Ab) were then investigated in these 13 pts. IL-10, IL-6 and IL-2 significantly enhanced NHL B cell proliferation (upplication) in respectively 13 (100%), 5 (38%) and 9 (69%) of 13 pts. IL-2 and IL-6 synergized with IL-10 to enhance lymphoma cell proliferation in 12 (92%) of 13 pts. These results show that IL-10, IL-6 and IL-2 are produced in vivo in all pts with NHL and exert additive effects on the in vivo proliferation of NHL B cells in autocrine or paracrine manner.

A HISOTOCHICAL ANALYSIS OF HTLV-1-ENCODED PROTEINS IN T-CELL MALIGNANT LYMMPHOMAS (T-MLL)

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In order to see whether HTLV-1 plays an oncogenic role in T-MLA, we analyzed HTLV-1-encoded proteins and their synthesis in T-MLA. TMLA are T-lymphocytes that are characterized by severe immunosuppression, DNA rearrangement of the TCR alpha and high expression levels of cytokines, while expression of HTLV-1 proteins are not evident. In the present study, we examined HTLV-1-encoded proteins by immunohistochemistry in five patients and evaluated whether HTLV-1 proteins play a role in the development of T-MLA. The antigens used were against p40tax and p54tax and on paraffin-seection of several cell lines with and without HTLV-1. Considering these conditions of the histochical detection of HTLV-1-encoded proteins, the following results and discussion were made. C192, a cell line of T-MLA examined were positive for the mRNA, the p40tax and the gp46tax in their lymphoma cells. In the high grade T-MLA, histio-pathologically defined adult T-cell leukemia/lymphoma (ATLL) type included many cases positive for the mRNA, the p40tax and the gp46tax. Subtypes of HTLV-1-related T-MLA were different from each other in the rate of cells positive for the mRNA, the p40tax and the gp46tax. Nuclear MoAb L4-1 stain was seen more often in low-grade T-MLA than in the ATLL type, suggesting that expression of lymphomagenesis would be in early and late phases of ATLL-pathogenesis. In the other hand, cell surface MoAb Gl2-1 stain was seen more often in the ATLL type than in low-grade T-MLA, suggesting virus reproduction.

2. Biology
3. Pathology


When reviewing the results obtained on flow cytometric studies of peripheral blood (PB), bone marrow (BM) and/or lymph nodes (LN) of 249 patients with B-cell chronic lymphoproliferative disorders, we found a CD5(+) immunophenotype on 153 cases. These cases had been classified as follows according to cytological and/or histological criteria: chronic lymphocytic leukemia and lymphoblastic lymphoma (143), centroblastic lymphoma (5) and lymphoplasasmacytic immunityoma (LPIC) (4).

The clinical, histological, cytological and flow cytometric features of the 4 cases of CD5(+) lymphoma which had been classified as LPIC were reviewed. All patients had peripheral and/ or retropertioneal adenomegalies and extensive BM infiltration at diagnosis. Two patients presented in lymph node stage and another patient developed a leukemic phase during the evolution of the disease. Extranodal sites were not involved, except for one patient (lung). A monoclonal serum IgM paraprotein was found in 3 out of 3 cases. One patient presented with autoimmune haemolytic anemia. Histology: diffuse proliferation of small and medium sized lymphoid and plasmacytoid cells, with or without Dutcher bodies. Cytology: Marked lymphocyte pleomorphism, small to medium sized lymphoid cells with irregular or "cleaved" nuclei, sometimes with inconspicuous nucleoli, scant to moderate sized and frequently basophilic cytoplasm. Flow cytometry: HLA-DR(+), CD19(+), CD20(+), CD22(+), CD23(+), CD38(+), FMC7(-), surface IgM (+), IgG, IgA, IgD(-). CD10(-), CD23(-), CD25(-).

According to the REAL classification these cases "should be regarded as a variant of B-CLL". However, we are convinced that CD5(+) LPIC should be considered a distinct entity with clinical and immunophenotypic features similar to that usually found on B-cell chronic lymphocytic leukemia / small lymphocytic lymphoma except for CD38 and CD23, cytological features similar to mantle cell lymphoma and histological features similar to conventional LPIC.

3. Pathology

HAPTIGLOBIN - RELATED PROTEIN (Hpr) AS A SERUM MARKER IN MALIGNANT LYMMPHOMA.

R. Epelbaum, C. Shalitin, R. Segal, C. Valansi, I. Arselan, D. Faraggi, M. Leviev, M. Ben-Shahar and N. Haim. Department of Oncology, Rambam Medical Center, and Department of Biology, Technion - Israel Institute of Technology, and Department of Statistics, Haifa University, Haifa, Israel.

A novel 21kDa protein, haptoglobin-related a chain (Hpr), has been recently found in sera of cancer patients (pts), utilizing an enzyme-linked immunosorbent assay (ELISA). We investigated serum Hpr levels in 88 pts with malignant lymphomas (male/female - 42/46; median age 48 years, range 18-87; non-Hodgkin’s lymphoma - 58; Hodgkin’s disease - 30) to evaluate its correlation with clinical and histologic features at presentation and its possible use as a tumor marker for patient outcome. Sera from 61 healthy volunteers served as normal controls. Serum Hpr levels in the lymphoma patients (median 430 x 10^3 U/ml, range 0 - 4000) were significantly higher than in the control group (median 68 x 10^3 U/ml, range 0 - 180) (p < 0.0001, Wilcoxon two-sample test). Higher median Hpr values were detected in pts with advanced disease (p < 0.013), “B” symptoms (p < 0.029) and in males (p = 0.053). There was also a significant correlation between Hpr and erythrocyte sedimentation rate (p = 0.028, Spearman rank’s correlation test). Serial levels showed a significant decrease of first Hpr values obtained after treatment in 41 pts (p < 0.0001, Wilcoxon signed-rank test for paired observations), 19 of whom achieved complete remission (CR). In the follow-up period, additional Hpr determinations were performed for 17 pts. Of 14 pts who maintained CR, Hpr levels remained low in 11, and increased in 3. Three pts eventually relapsed, and showed increased Hpr levels at the time of relapse. In conclusion, serum Hpr is a new biologic serum tumor marker of potential use in the clinical setting of lymphoma.

Supported by Chemotex Technologies Ltd.

3.17

PROGNOSTIC FACTORS IN LOW GRADE NHL (A MULTIVARIATE ANALYSIS).

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During the period between January 1970 and December 1989 inclusive, 278 newly diagnosed low grade NHL patients were treated and followed up at the RMIH, England. The median survival and PFS was 8.75 years and 2.5 years respectively (median follow-up: 8 years). By univariate analysis, age (60 years), stage (III and IV), more than 2 sites of disease, extranodal disease, anaemia, B-symptoms, bone marrow involvement, ESR 4 mm/h and chemotherapy treatment were adverse prognostic factors affecting survival. By multivariate analysis more than 2 sites of disease, age (60 years) and anaemia, remained as significant adverse prognostic factors. For PFS old age, advanced stage, more than 2 sites of disease, extranodal disease, bone marrow involvement, liver involvement, anaemia and chemotherapy treatment were univariate adverse prognostic factors. By multivariate analysis more than 2 sites of disease and extranodal disease remained significant.

3.18

HAPTIGLOBIN - RELATED PROTEIN (Hpr) AS A SERUM MARKER IN MALIGNANT LYMMPHOMA.

R. Epelbaum, C. Shalitin, R. Segal, C. Valansi, I. Arselan, D. Faraggi, M. Leviev, M. Ben-Shahar and N. Haim. Department of Oncology, Rambam Medical Center, and Department of Biology, Technion - Israel Institute of Technology, and Department of Statistics, Haifa University, Haifa, Israel.

A novel 21kDa protein, haptoglobin-related a chain (Hpr), has been recently found in sera of cancer patients (pts), utilizing an enzyme-linked immunosorbent assay (ELISA). We investigated serum Hpr levels in 88 pts with malignant lymphomas (male/female - 42/46; median age 48 years, range 18-87; non-Hodgkin’s lymphoma - 58; Hodgkin’s disease - 30) to evaluate its correlation with clinical and histologic features at presentation and its possible use as a tumor marker for patient outcome. Sera from 61 healthy volunteers served as normal controls. Serum Hpr levels in the lymphoma patients (median 430 x 10^3 U/ml, range 0 - 4000) were significantly higher than in the control group (median 68 x 10^3 U/ml, range 0 - 180) (p < 0.0001, Wilcoxon two-sample test). Higher median Hpr values were detected in pts with advanced disease (p < 0.013), “B” symptoms (p < 0.029) and in males (p = 0.053). There was also a significant correlation between Hpr and erythrocyte sedimentation rate (p = 0.028, Spearman rank’s correlation test). Serial levels showed a significant decrease of first Hpr values obtained after treatment in 41 pts (p < 0.0001, Wilcoxon signed-rank test for paired observations), 19 of whom achieved complete remission (CR). In the follow-up period, additional Hpr determinations were performed for 17 pts. Of 14 pts who maintained CR, Hpr levels remained low in 11, and increased in 3. Three pts eventually relapsed, and showed increased Hpr levels at the time of relapse. In conclusion, serum Hpr is a new biologic serum tumor marker of potential use in the clinical setting of lymphoma.

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3.19


DESAIBENS B, CAPIOUD J-C, GOURELLEUX V, PUYTEN V, GARIDR R, CLAISSIE J-F, PRIN L - CHU AMIENS - 80054 AMIENS - FRANCE.

Seric levels of soluble IL2 receptor (sCD25) and soluble low-affinity Ig receptor (sCD32) were measured by ELISA kits. Normal values range respectively from 529 to 913 UI/l and from less than 0.15 to 3.3 UI/l Among our 109 patients (pts) with a newly diagnosed B-CLL, mean levels were 2,002 ± 2,149 UI/l for sCD25 and 244 ± 145 UI/l for sCD32. Median values were respectively 1,053 and 27 UI/l. On January 1996, the median follow-up time is 4 years and we find that the best cut-values are 2,000 UI/l for sCD25 and 30 UI/l for sCD32.

Because of a strong correlation between sCD25 and sCD32 levels and all other prognostic parameters of B-CLL (number of lymphoid areas; blood and medullary lymphocytes; bone marrow biopsy; hemoglobin and platelets counts ...), levels are quite different among pts with stage A and pts with stage B + C.

However sCD25 and sCD32 levels keep a strong prognostic value among the 90 stages A:

<table>
<thead>
<tr>
<th>Survival</th>
<th>Rate</th>
<th>B + C (19 pts)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCD25</td>
<td>1,449 ± 1,664 UI/l</td>
<td>4,030 ± 2,280 UI/l</td>
<td>&lt; 10^-8</td>
</tr>
<tr>
<td>sCD32</td>
<td>849 ± 541 UI/l</td>
<td>5,013 ± 6,613 UI/l</td>
<td>&lt; 10^-4</td>
</tr>
</tbody>
</table>

We consider the prognostic importance of sCD25 and sCD32 levels in B-CLL but 2 points are to be debited: the determination of the best cut-values and the true importance of these new biological prognostic factors in opposite to the clinical factors such as Binet staging.

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3. Pathology
CA-125: A NEW TOOL PREDICTIVE OF RESPONSE IN LYMPHOMAS?

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The occasional finding of high CA-125 serum levels in some pts with lymphomas undergoing staging work-up, prompted us to examine and investigate its possible clinical and biological significance. Aim, 1) to evaluate the frequency of abnormal CA-125 levels in a large series of consecutive lymphoma pts, to define the correlation between pretreatment CA-125 serum levels and the main patient features; 2) to determine whether abnormal pretreatment values of CA-125 significantly correlate with the probability of Complete Response (CR). Methods, CA-125 serum levels were assessed, by using the new Centocor Ca-125II IMIA assay, before first line or salvage treatment in pts with either newly diagnosed or recurrent lymphomas. A multivariate logistic analysis was performed to verify whether pretreatment high CA-125 levels were independently associated with a lower CR rate. Results, 108 consecutive patients were evaluated (pts).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total</th>
<th>HD</th>
<th>NHL</th>
<th>Newly diagnosed</th>
<th>Recurrent</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR%</td>
<td></td>
<td>108</td>
<td>25(23%)</td>
<td>83(77%)</td>
<td>22(21%)</td>
<td>15(25%)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Abnormal CA-125 significantly correlated with the abnormal LDH and bulky disease in both newly diagnosed and recurrent pts. Besides, a significantly higher probability of abnormal CA-125 was found in abnormal stage and recurrent disease.

The correlation with CR showed:

<table>
<thead>
<tr>
<th>CA-125 levels</th>
<th>Abnormal normal</th>
<th>Abnormal normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR%</td>
<td>15/50(30%)</td>
<td>48/35(40%)</td>
</tr>
<tr>
<td>p value</td>
<td>0.0093</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Results as multivariate analyses on newly diagnosed pts were as follows:

<table>
<thead>
<tr>
<th>CR%</th>
<th>R.C. &lt; 5E</th>
<th>R.Risk</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>≥ 65 yrs</td>
<td>53</td>
<td>1.84 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>&lt; 65 yrs</td>
<td>59</td>
<td>1.00 ± 0.65</td>
</tr>
<tr>
<td>CA-125 &lt; 35 RU</td>
<td>50</td>
<td>1.18 ± 0.64</td>
<td>3.20 (95-17.7)</td>
</tr>
</tbody>
</table>

Conclusions: Abnormal CA-125 serum levels were found in about a half of pts with a half of pts with lymphoma and were associated with a significant reduction of CR rate in both newly diagnosed and recurrent pts. On the basis of these preliminary data, age ≥ 65 yrs, abnormal pretreatment CA-125 and LDH levels are the variables which better predict a poor CR rate, in both newly and recurrent patients.

GENETIC LESIONS OF NON-HODGKIN’S LYMPHOMA (NHL). ANALYSIS OF BCL1, BCL2, BCL6, C-MYC, 11q23/MLL AND P16 GENES IN 130 CASES


Bcl-2: 104/120 patients showed bcl-2 gene rearrangements (63% in follicular NHL and 31% in large cell NHL). The breakpoint region affected was mbr in 30 cases (88%) and mcr or vnr in 3 and 3 cases, respectively. A double rearrangement mbr-b was detected in 2 cases. No changes in bcl-2 were found in other subtypes, with the exception of two small lymphocytic and one Burkitt-like NHL.

Bcl-6: A total of 89/159 (54%) samples exhibited bcl-6 rearrangements. 5 patients had diffuse large B-cell lymphoma, while the remaining 5 patients were follicular NHL.

C-myc: c-myc protooncogene was rearranged in 5 patients, all cases being Burkitt’s lymphomas, excepting one case of mantle-cell NHL.

11q23: Abnormalities of 11q23/MLL gene were not found in any patient.

P16: Alterations of the P16 tumor suppressor gene occurred in 5 out of 63 (8%) patients. Three were homologous deletions and two rearrangements. 4 patients were follicular NHL and one, with a monochromatic translocation, had Sezary’s syndrome.

Our study shows that abnormalities of protooncogenes and tumor suppressor genes are frequent in NHL and are often associated with a particular histologic subtype, although not always specific for a given rearrangement. (For example, 11q23/MLL are not restricted to high grade malignancies as previously suggested, but also occur in low-grade lymphomas - follicular subtype).

Stage related differences of cell growth in Non-Hodgkin's lymphomas.


The natural history of most animal tumors follows the Gompertz function, which exhibit a progression decrease of growth. May the proliferative activity of Non-Hodgkin's lymphomas (NHL) change as a function of stage (Ann Arbor)?

One hundred and five cases of diffuse NHL of various types and subtypes were collected. Malignant, apolitic and Tumor indices (respectively Mi, Al and Tmx+Al) were established in each case. In 76 of 105 cases, the percentages of Ki-67+ cells and of bcl-2+ cells were also determined.

When all cases were taken together, localized stage IA-II (21 cases) exhibited clearly higher Mi, Al and Tm, as well as significantly lower percentages of bcl-2+ cells, then disseminated stages II-IV (58 cases). A similar pattern was found when only high grade (Kiel classification) lymphomas (51 cases) were evaluated. Low grade NHL (54 cases) showed analogous stage-dependent characteristics, with the exception of median percentages of bcl-2+ cells which remained the same in all stages. Ki-67+ cell fraction was markedly higher in high grade NHL lymphomas, and also correlated with the so-called growth fraction do not correlate very closely.

Our findings are consistent with the notion that dissemination of diffusely growing NHL is usually associated with a reduced cell turnover and, in high grade lymphomas, with the generation of longer-living cells. These findings may open novel therapeutic tools in the management of malignant lymphomas which may depend on the stage.
DIFFUSE LARGE B-CELL LYMPHOMA: DOES SUBGROUPING CORRELATES WITH SPECIFIC CYTGENETIC FINDINGS?
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According to the REAL subclassification, diffuse large B-cell lymphomas (DLBCL) are divided into classical and additional studies to be needed to identify possible specific entities within it. In order to identify such entities, we used the WHO classification and correlated these with the clinical picture. We identified 69 cases diagnosed as DLBCL. The cytogenetic analysis was performed on frozen sections and with successful abnormal cytogenetic analysis (61 cases performed at diagnosis and 8 cases at relapse).

The identification of morphological groups was based on H&E sections and was conducted without knowledge of the cytogenetic results. Group 1 comprised cases composed predominantly of large cleaved cells (15 cases); in 5 of these a vague nodular pattern could be recognized. Group 2 the lymphoma was composed of large non cleaved cells (28 cases); in 5 cases a monotonous proliferation was found, while in 12 cases nuclear pleomorphism was noted. In the remaining 11 cases a prominent stromal reaction was seen either associated with a large number of necrotic cells and necrotic activity (6 cases) or with rare neoplastic cells (5 cases). Group 3 was composed of large B cells with cytological features reminiscent of monocytoid B-cells (cases). Group 4 comprised 6 cases all characterized by a pronounced plasmacytic differentiation. The remaining 12 cases were recognized as anaplastic large B-cell (3 cases), small non cleaved (3 cases), multilobated (2 cases) and cases not further subclassified. Cytogenetic analysis revealed that 6q (20 cases) and 16q trisomy (10 cases) and/or +18 (20 cases) were present in all morphological subgroups, although the latter was found in 9/8 cases of group 2, 8 of 14 cases (18) was observed in all but one subgroup belonging to group 1. When cases were grouped based on their karyotype, no morphological similarities were found within these except for cases carrying t(14;18). In summary, although several subgroups may be identified, with our present data we can document only one subgroup, composed of large cleaved cells, which is associated to a specific cytogenetic abnormality.

ACUTE B-CELL LEUKEMIA/LYMPHOMA WITH (14;18) TRANSLOCATION
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The (14;18) translocation is found in 70 to 90% of the patients with follicular NHL. In this clinical case, the disease is often indolent but progression into aggressive lymphomas may occur. The same translocation has been reported in 2 cases of diffuse large B-cell lymphoma which have an aggressive course. We have observed three cases of large B-cell lymphoma with initial leukemic phase and a t(14;18). Patient 1. A 27-year-old woman presented with generalized lymphadenopathy, neutropenia and pancytopenia. The WBC count was 35x10^9 with 80% blasts, the test for HIV antibodies was negative. The bone marrow was involved. Lymph node biopsy showed a large B-cell lymphoma. Immunophenotype showed B-cell associated antigens and surface IgD. Positive. Cytogenetic analysis demonstrated a complex chromosomal abnormalities with (14;18) translocation. Despite intensive chemotherapy the patient died 6 months after initial diagnosis.
Patient 2. A 38-year-old woman presented with axillary lymphadenopathy, neutropenia and a right abdominal mass. Mediastinum, kidney and bone marrow were also involved. The WBC count was 72x10^9 with 61% blasts. A test for HIV antibodies was negative. The diagnosis of large B-cell lymphoma was done. Cytogenetic analysis showed a t(14;18) and additional abnormalities. The patient died 6 months after the diagnosis.
Patient 3. A 50-year-old patient presented with abdominal pain. Clinical examination showed multiple enlarged lymph nodes and CT-scan of the abdomen revealed a large retroperitoneal mass. The WBC count was 12.3x10^9. Lymph node biopsy showed a diffuse large cell lymphoma. The bone marrow was involved with 85% blast cells. Immunophenotype was the same as patient 1. Cytogenetic analysis showed a complex translocation t(14;18)Xq24;32;21) and t(14;X)(q27;p13). The patient died 5 days after the onset of the chemotherapy.
DNA analysis showed a BCL-2 rearrangement in patient 2 and 3, but it could not be demonstrated in patient 1, either by PCR or southern blot analysis. BCL-2 was rearranged only in patient 3. c-myc rearrangement was found in all three patients. As described before, these patients had a particular acute clinical features, massive bone marrow involvement by blasts cells with a mature B-cell phenotype. Additional oncogenic events for BCL-2 rearrangement as c-myc activation could explain this aggressive behaviour.
Cytologic characteristics define karyotypically homogeneous subgroups in diffuse B large-cell lymphomas (DLCL).

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Lymphoma Research Group, Centre National de Recherche en Biologie (CNB) () 2017.30947 GINECO.

Introduction: During the past ten years many efforts have been made to describe among DLCL some entities based on multidisciplinary studies like in small cell lymphomas, but it has been concluded in the REAL that "it is impractical to subclassify these tumors with the current histological, immunological & genetic data". All these studies which have tried to find multidisciplinary correlations are based on inference tests. In this study we have used statistical methods to the Lymphocyte Content Analysis: PCA which led us to describe on cytological criteria a non random cytogenetic pattern in DLCL.

Method: 170 patients (pts) were selected in ten years (1985-94). inclusion criteria were B immunoblastic (IB) or centroblastic lymphoma (REAL classification). Follicular lymphomas were excluded. A detailed cytologic study was then performed on GIEMSA lymph node aspirates (ref 1). 4 criteria characterized the smears defining 13 quantitative cytologic variables (V1 to V13) (Table 1): number of cells (20×), 10× fibroblasts, 20× nuclei, 250× nuclei. The correlation matrix among these 13 variables was performed (Table 2). We defined the parameters of the 9 areas, each defined by a single or a combination of these 13 variables.

Results: A PAC: cumulative evaluation of the two first axes were 38%. Pts with similar cytological characteristics were located close to each other on this plane. All binomial t test to compare the cytological profiles with a predominance of cells 13-17×, diameter (average: 50% range: 5-65%), with a medium median (93%) of the cells, of the 5-100%, of standards high medium and high in 92% of the cells (70-100%) and 0 to 2% microvesicles. They were all IL2LM and all expressed MHC or MHC II. Clinical they were all III or IV (50%) with extra-nodal sites (2 for 5 for). Klöppel karyotype was usually complex showing 1 to 8 out of the 13 already mentioned breakpoints without 18q21. The 20 pts with 18q21 were distributed opposite to the 13 pts with 18q21, split by the first axis. In parallel the multivariate analysis had confirmed the non pertinent character of immunologic markers.

Conclusions: The heterogeneous group of large B cell lymphomas we have described a possible organisation of some patients with cytologic and cytogenetic data.


INTERLEUKIN 6 (IL6) IN NON-HODGKIN'S LYMPHOMA (NHL): ANALYSIS OF SERUM LEVELS AT DIFFERENT PHASES OF THE DISEASE.

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IL6 is an immunomodulatory cytokine that may have an important role in NHL progression: The prognostic value of IL6 serum levels in NHL has been recently pointed out. In a series of 110 patients with NHL (M: 1:3; median age: 53 years), 177 samples obtained at different phases of the disease (33 at diagnosis, 90 after achieving complete remission (CR), 22 at relapse, and 32 in disease progression) were analyzed in order to describe the evolution of serum IL6, to correlate it with clinicopathological variables, and to assess its prognostic value. IL6 levels were measured by ELISA, using a group of 25 healthy people as control population (normal value: < 5 pg/ml). The main results are detailed in the table:

<table>
<thead>
<tr>
<th>DIAGNOSIS (n=33)</th>
<th>CR (n=90)</th>
<th>RELAPSE (n=22)</th>
<th>PROGRESSION (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVERALL SERIES (n=177)</td>
<td>11.7±4.7</td>
<td>5.6±5.5</td>
<td>20.7±4.1</td>
</tr>
<tr>
<td>LOW-GRAD (n=67)</td>
<td>7.8±6.5</td>
<td>4.4±5.5</td>
<td>15.3±4.0</td>
</tr>
<tr>
<td>INTERN/HIGH GRAD (n=110)</td>
<td>13.7±18</td>
<td>6.5±11*</td>
<td>25.2±6.3</td>
</tr>
</tbody>
</table>

*p<0.05 vs. diagnosis
No significant differences were found according to histologic subtype (low vs intermediate/high grade).
In the 33 cases analyzed at diagnosis, patients with high serum IL6 levels had more frequently B-symptoms, poor performance status, anemia and lymphocytopenia (p<0.05 in all cases). No significant correlation was found between IL6 and response to therapy. In the group of CR patients, serum IL6 did not correlate with the risk of relapse. However, patients with elevated serum IL6 at diagnosis had shorter survival than the others (2 year survival: 62.5% and 91%, respectively, p<0.05). In conclusion, these results emphasize that serum IL6 can be an important parameter at the initial and eventual evaluation of NHL patients.

SERUM TUMOR NECROSIS FACTOR (TNF): A USEFUL PARAMETER FOR PREDICTING RELAPSE AND OUTCOME IN NON-HODGKIN'S LYMPHOMA (NHL).

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TNF is a cytokine with immunomodulatory properties that could have pathogenic and prognostic significance in NHL. In a series of 119 patients with NHL (M: 1:1.71; median age: 53 years), 177 samples obtained at different phases of the disease, in order to analyze the evolution of serum TNF, it correlate to the main initial variables, and to assess its prognostic value for response, relapse and survival. Serum TNF levels were measured by ELISA method, using a group of 25 healthy people as control population (normal value: < 20 pg/ml).

Serum TNF levels (median ± SD) were 66.2±102 pg/ml at diagnosis (33 cases), 14.6±17 at complete remission (CR) (30 cases; p=0.001 vs. diagnosis), 47.8±62 at relapse (22 cases; p=0.001 vs. CR) and 170.6±144 at progression (32 cases; p=0.001 vs. CR). When this analysis was performed separately for low and intermediate/high grade NHL, the differences among the phases of the disease were similar to those described above, with no differences according to histologic subtype.

In the 33 pts in which TNF was measured at diagnosis, patients with elevated serum TNF had more frequently B-symptoms, poor performance status, advanced stage, bone marrow infiltration (p<0.05 for all these variables), high LDH and 02 microglobuline (p=0.001), low WBC, lymphocyte (p=0.001) and platelet (p=0.01) counts. No significant relationship was found between TNF and CR achievement. Patients with high TNF at diagnosis had a shorter survival (2-year survival: 69% vs. 90%; p=0.01). Finally, the risk of relapse was analyzed in 50 patients in CR in whom serum TNF was obtained within the 6 months from CR achievement. Twelve of 20 patients with high TNF relapsed in contrast with 6 of 30 patients with normal TNF (p=0.01). No patients with serum TNF > 8 pg/ml relapsed during the follow-up. The actuarial risk of relapse was 21% (95%CI: 3-37) and 48% (95%CI: 28-68) for patients with normal and elevated serum TNF, respectively, at 18 months of the sample analysis (p=0.05). In conclusion, TNF might be a useful parameter to predict the outcome of NHL patients.

THE PROGNOSTIC SIGNIFICANCE OF PROLIFERATING CELL NUCLEAR ANTIGENS AgNORs, PCNA and p53 IN NON-HODGKIN'S LYMPHOMAS.

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Application of the colloidal silver method for demonstration of interphase nuclear organizer regions (AgNORs) and the monoclonal antibody PC-10 for recognition of proliferating cell nuclear antigen (PCNA) have both been shown to reflect the proliferative state of non-Hodgkin's lymphomas (NHL). On the other hand, the protein product of p53 gene plays a critical role in the control of cell proliferation acting as a negative regulator at the G1-S phase transition, whereas mutant p53 protein is associated with cell proliferation and tumor progression. The prognostic value of the immunohistochemical expression of PCNA and p53 and the mean number of AgNORs per nucleus (AgNORs score), in relation to classical clinicopathological parameters, was assessed in 91 patients with NHL, using univariate and multivariate analysis. Univariate analysis showed that histological type and grade, clinical stage, the type of chemotherapy, p53 labelling index (LI), PC-10 LI and AgNORs score were significantly related to overall survival: the higher PC-10 LI, the p53 LI and the AgNORs score, the shorter the survival time. However, only two parameters -histological grade and p53 LI influenced disease-free survival. In multivariate analysis, clinical stage, PC-10 LI, AgNORs score and bone marrow infiltration predicted overall survival independently. In that order. Histological grade was the only independent predictor of disease-free survival, whereas PC-10 LI and p53 LI were the independent predictors of post-relapse survival. These results indicate that AgNORs and PCNA are better predictors of overall survival than histological grade. The latter appears to reflect cell proliferation data only in relation to disease-free survival.
Presence of resistant forms in non-Hodgkin's lymphoma (NL) is generally attributed to availability of quite large number of tumour cells - lymphocytes which are insensitive to chemotheraphy. It may be assumed that population of these cells differs from other lymphocytes by characteristic changes in their structure. We study electroconductivity (E) of lymphocytes separated from blood of patients with NL. Registration of E were conducted at 102 - 107 Hz, as it was considered that changes in E at low frequency (102 - 105 Hz) are mainly caused by mobility of cell surface charged groups, while electroosmosis properties of cellular structures supposed to be responsible for changes in E at high frequency (106 - 107 Hz). At low frequency 39 out of 46 patients with NL exhibit decrease in lymphocytes E compared to normal cells, while at high frequency impairment in E was found for 35 patients. Additional studies pointed out that all these changes must be attributed to labilization of lymphocytes plasma membrane structure. These data testified to high sensitivity lymphocytes E to the disorders of cell structure in NL. But the most important seems that lymphocytes of 18 patients from the group with high resistance to chemotherapy exhibited characteristic changes in their low frequency which can be served as an additional criterion for prognosis of resistant forms in NL.

HIV-POSITIVE CD30+ ANAPLASTIC LARGE CELL LYMPHOMA (HIV+ CD30+ ALCL+): CLINICAL AND PATHOLOGICAL FEATURES.

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Aim: To compare the clinical and pathological features of 16 HIV+CD30+ALCL patients (9 M, 7F, mean age 39.9 yrs) to those of 28 HIV-CD30- NHL (23 M, 5 F, mean age 51.7 yrs).

Results: The incidence of ALCL in 28 consecutive patients with systemic HIV+ NHL was much higher (20%) than that for 9010 HIV+CD30+ALCL patients had stage IV at diagnosis and none had nodal presentation; median CD4 count was 53 x 10^9/L (range 10-130); opportunistic infections (OI) were present in 6/18; ECOG-PS was > 2 in 6 cases. Among 28 HIV+CD30- NHL patients, 8, 3, 17 were in stage I, III and IV respectively. ECOG-PS was > 2 in 11/28; median CD4 107 x 10^9/L (range 2-768), OI occurred in 10/28 cases. In our study group HIV+CD30+ ALCL differed from HIV+CD30- NHL for the increased frequency of lung (40% vs 21%), bone marrow (21% vs 18%) and gastrointestinal (40% vs 25%) involvement. In HIV+CD30+ ALCL the skin was involved, along with multiorgan localization, only in one patient; none of the patients with long involvement had concomitant mediastinal masses; no correlation between HIV-related subtype and presence of bulky mediastinal mass was found. Among our HIV+CD30+ALCL patients, 5 had B phenotype, 4 null and 1 T phenotype; in HIV+CD30- NHL patients, phenotype was B in 25 cases, T in 2 and null in 1 case. EBV genome was studied in only HIV+CD30+ALCL and was present in 3/10 patients. S10 HIV+CD30+ALCL and 12/28 HIV+CD30- NHL were treated with standard dose CHOP. In CD30+ group one patient is still alive at 5 months from diagnosis; in the remaining 9 patients death was secondary to lymphoma progression in 5 cases and to both lymphoma progression and OI in 4 cases. In CD30- group 1 patient was lost to follow-up, 4 are still alive and in CR at 37, 76, 10 and 5 months from diagnosis; death was secondary to lymphoma progression in 39%, OI in 30%, both in 22%, other causes in 9% of cases. Median survival was 84 days (range 3-270) in HIV+CD30+ALCL and 188 days (range 7-1650) in HIV+CD30- NHL.

Conclusions: Probably, the severe immunodepression due to HIV infection determines, more than any other factor, the clinical features of HIV+ ALCL, making them very similar to other high grade systemic HIV-NHLs.
BILATERAL BONE MARROW BIOPSY IN LOW GRADE NON-HODGKIN'S LYMPHOMA

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Bone marrow biopsy (BMB) is a routine investigation in the diagnosis and staging of non-Hodgkin's lymphoma (NHL). Bone marrow involvement is correlated with prognosis and often may be focal. For these reasons it was suggested to perform bilateral biopsy to reduce the false negatives. In literature there are some reports showing that the bilateral biopsy increase of about 30-50% the diagnosis of bone marrow infiltration particular in intermediate or high grade NHL.

We have analized from 1992 72 bilateral BMB in 61 low grade NHL patients, 57% male and 43% female, 85% A, 9% B, 62% CD20 lymph node histology according to Working Formulation. 41 were bilateral BMB staging, 24 were restaging BMB and 7 were BMB of relapsed patients. All biopsies were done using 1% Xylocaine local anesthesia and the specimens were placed in standard formal membrane Jr Kit (Parafix) and processed for morphological assessment according to routine techniques.

Sections were cut at 1.5 μm using Reichert-Jung microtome. The median height of the biopsy specimens from the right and the left posterior superior iliac crests was 15.9 mm (range 8-30 mm) and 17.8 mm (range 8-31 mm), respectively. The median preserved marrow spaces were respectively 6.1 (range 4-11 spaces) and 5.8 (range 3-10 spaces) for right and left biopsies. The marrow cellularity was 40.2% (range 20-60%) for right biopsies and 42.1% (range 20-70%) for left biopsies. In our study no cases had unilateral disease, the right and the left trephine biopsy specimens were concordant whether for involvement by malignancy or not and the positive ones showed the same pattern of marrow disease. 29% had BMB bilaterally positive at staging, 20 positive stage patients were restaged with bilateral biopsy after chemotheraphy; 5 (25%) continued to have bilateral marrow infiltration and the other 15 (75%) had no bilateral marrow infiltration. Three of seven relapsed patients have marrow infiltration and it was bilateral infiltration.

In 23 patients was performed PCR study on marrow sample and it was found that there were exact agreement between right and left sample in all cases and in particular in 14 of these patients (61%) morphology and molecular biology were identical. The remaining 9 cases biology was bilateral positive and morphology was bilateral negative.

We can conclude, according to these data, that bilateral biopsy in low grade NHL doesn't modify the stage and doesn't reduce the false negative. We can advise the bilateral BMB with a unilateral one but of adequate dimension.

NHL WITH PRIMARY PRESENTATION IN THE SPLEEN

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Primary splenic lymphomas are uncommon and constitute 1-2% of all the malignant lymphomas.

We retrospectively analyzed 14 patients (5 men, 9 women) with NHL, who had primary presentation of the disease in the spleen. Their median age was 62 (range 45-75). The most common presenting symptoms were symptoms of anaemia, fever, malaise, weight loss and pain in the spleen. All had splenomegaly, four had also hepatomegaly, but no one had palpable lymphadenopathy. From the peripheral blood anaemia, thrombocytopenia, lymphocytosis and pancytopenia were observed. The bone marrow was involved in 7 of the 14 patients. CT scan of the chest and abdomen was negative for lymph nodes, in all patients.

All 14 patients underwent laparotomy with splenectomy. The diagnosis was made from the spleen biopsy. Small lymphocytic lymphoma was the most frequent histologic type, followed by large cell lymphoma, mixed cell lymphoma and marginal cell lymphoma.

Twelve patients received chemotherapy (Chlorambucil+prednisolone, CHOP, C E D P, MACO-B). Nine patients remain in complete remission 18 to 132 months after splenectomy. Tree patients are alive 6 to 8 months, still under chemotherapy. Two patients died, because of sepsis, 6 and 12 months after splenectomy.

PRIMARY ANAPLASTIC LARGE CELL LYMPHOMAS FROM A SINGLE CENTRE INSTITUTION: CHARACTERISTICS AND PROGNOSIS OF 61 CASES COMPARED TO OTHER HIGH GRADE LYMPHOMAS.

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Sixtyone patients with primary anaplastic large cell lymphomas (ALCL) were compared to a selected group of 192 patients with centroblastic (157) or immunoblastic (35) lymphomas. All patients were diagnosed and treated at the same institution from 1986 till 1993. Clinically, ALCL patients had more often B symptoms, enlarged peripheral lymph nodes and mediastinal involvement compared to centroblastic immunoblastic patients, who were older and more often showed extranodal disease. There were no differences concerning sex, bulky disease, spleen enlargement or bone marrow involvement, nor concerning the fraction of patients with stage III/IV disease in the two groups.

All the patients were treated according to the chemotherapy and radiotherapy protocols of the institution. The estimated 3 year overall survival for the ALCL group was 61% compared to 51% for the centroblastic/immunoblastic group. This difference was not statistically significant.

Univariate analysis in the ALCL group revealed that advanced stage (III elf IV) and high LD value were adverse prognostic variables, while in the cbl/bib group systemic symptoms and high erythrocyte sedimentation rate were additional adverse prognostic variables.

Multivariate analysis of survival in all patients showed that a high serum LD value, advanced stage (III+IV), systemic symptoms and to a lesser extent older age, were adverse prognostic variables. A diagnosis of anaplastic large cell lymphoma was not an additional adverse prognostic variable.

In conclusion, anaplastic large cell lymphomas affect a younger population of patients, have a different clinical presentation, but a similar prognosis compared to patients with centroblastic or immunoblastic lymphomas.

MARGINAL ZONE B-CELL LYMPHOMA WITH CUTANEOUS ONSET: AN AGGRESSIVE CLINICAL VARIANT?


The histology and clinical characteristics of the Marginal zone B-cell lymphoma (MZB NHL) have been described in recent years. This tumor has two subtypes: the first involving mainly extranodal sites (MALT type), the second with more relevant nodal disease. The different localization could be related to the hunning pattern of the lymphoma cells. We report two cases of MZB NHL, characterized by primitive cutaneous or subcutaneous lesions with successive recurrent diffuse lymph node involvement. Patient No. 1: Male, aged 61, presented from 6 months before our 1st observation subcutaneous nodules on the back and on the right arm of the patient with right axillary and left inguinal lymph node enlargement. A MZB NHL was diagnosed by skin and lymph node biopsy. The CT scan showed retroperitoneal and left iliac lymphadenopathies. After a 2 month treatment with chlorambucil + IFN, because of the onset of a systemic symptoms with lymph node enlargement, the patient was started on ProMACE-CyBOA (3 cycles) with partial and temporary response. For this reason this non-cross-resistant treatment (MCMA) was administered for 3 cycles with fairly good control of the disease for some months. The further increase of the left iliac, inguinal lymphadenopathies with the onset of deep vein thrombosis of the left leg led us to begin a treatment with fludarabine. Patient No. 2: Man, 54 years, with diffuse papulopustular skin lesions prevalently localized to the face, the neck and the trunk onset about 2 years before, came to our observation for the enlargement of right inguinal lymph nodes. At the moment the patient presented also mild anaemia, thrombocytopenia, microcytoblastemia and procamia, mild renal failure and monoclonal gammapathy (lgA). CT scan showed also diffuse thoricic and abdominal lymphadenopathies, without evident kidney infiltration. Lymph node, skin and bone marrow biopsies allowed the diagnosis of MZB NHL. The patient was partially responsive to 5 cycles of ProMACE-CyBOA, so that a subcutaneous therapy (MCMA) with PBSC collection was started and after 3 cycles a PBSC transplantation was performed. Now the patient is CR 4 months after the PBST, without any sign of procarcin, leucocyte, renal failure and monoclonal gammapathy. Our 2 cases are characterized by the cutaneous and subcutaneous primitive onset of the disease followed during some months by lymph node involvement associated with a rapid progression of the disease consistent with the indolent clinical course commonly described for MZB NHL. The 1st patient was totally unresponsive to the treatment, while the 2nd obtained the CR only after subsequent chemotherapy. Even though we have observed only two cases, the clinical analogies of these patients invite us to suspect that a multidiscetaneous cutaneous involvement at the onset of the disease in MZB NHL could be associated with very aggressive course of the lymphoma.
MESANGIOPROLIFERATIVE GLOMERULONEPHRITIS IN PRIMARY RENAL LYMPHOMA - A CASE REPORT
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Introduction: Primary Non-Hodgkin-Lymphoma (NHL) of one or both kidneys without any other nodal or extranodal involvement is a rare entity. We present a unique case of primary renal lymphoma associated with metachronic glomerulonephritis.

Case report: A 27-year old male developed nonoliguric renal failure. Biopsy of the kidneys showed infiltration by a centroblastic NHL. Staging procedures including CT scanning of thorax and abdomen, bone marrow biopsy and laparoscopy revealed no further manifestation of NHL. The patient received 6 courses of CHOP chemotherapy and achieved complete remission. During annual follow-ups the patient remained in complete remission and creatinine levels ranged between 1.9 and 2.1 mg/dl. 8 years after his first presentation the patient developed acute oliguric renal failure with nephrotic syndrome. A diagnosis of mesangioproliferative glomerulonephritis was made on biopsy of the left kidney. Chronic hemodialysis was required until after 5 years cadaver kidney transplantation was successfully performed.

Conclusion: The occurrence of 2 diseases in the same organ raises the question of causal relationship. Although the association of NHL and glomerulonephritis has been described several times before, to our knowledge, this is the first report of glomerulonephritis in a primary renal lymphoma.

FOLLICULAR CUTANEOUS T-CELL LYMPHOMA WITHOUT MUCINOSIS. REPORT OF 9 CASES.
Beylot-Barry M1, Vergier B1, Beylot C1, DelMarecami A1, DeLamauy M1, DeMuret A2, Vaillant L2, Tortel E2, Grange F2, Bagot M2, Laroche L2, Weschler J2, and the French Study Group of Cutaneous Lymphomas. From the Departments of Dermatology and Pathology, Universities of Bordeaux (1), of Tours (2), of Colmar (3), of Paris Creteil (4) and Bobigny (6), France.

During mycosis fungoides (MF), follicular manifestations without mucinosis are rare, with only 14 cases reported. Their clinical and histological diagnosis may be difficult. The clinical course, histopathological and immunohistochemical features of 9 cases are described.

Follicular lesions were present before MF (n = 3), at the onset of MF (n = 3), or during a relapse (n = 3). The 3 patients with late follicular lesions were previously treated by PUVA-therapy, electrontherapy or Chlorimethene. Follicular lesions especially arose in areas where hair follicles are abundant as face, thighs or buttocks. While congo and epithelial cysts were most frequent (n = 5), follicular lesions can be sometimes deceptive with spinulositis keratosus, or lesions improved or disappeared with lymphoma therapy alone (n = 4) or associated with isothorin (n = 3). In case they disappeared spontaneously and persisted in another patient in terminal phase of his lymphoma.

Cases of histopathological diagnosis were pilomatricoma of the infiltrate with minor alteration of follicular walls. Infiltrate was monomorphic, composed of seanzarriform CD4 + lymphocytes. Pilomatricoma, then decreased around epidermal cysts were detected on consecutive biopsies of a same patient. Keratinocytes ICAM-1 expression was observed in the hair follicle bulb in front of LFA-1 positive folliculotropic infiltrate but not in the epidermis in cases of follicular mucinosis and in folliculotropic lesions or normal follicles. Our findings indicate the role of adhesion molecules ICAM-1 and LFA-1 in the pilomatricoma leading to mechanical obstruction by tumoral cells, hyperkeratosis and then cyst formation. It remains to determine if ICAM-1 expression is the cause or the consequence of pilomatricoma.

MALIGNANT LYMPHOMA IN JAPANESE HTLV-I NONENDEMIC AREA, BASED ON THE REAL CLASSIFICATION.
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A clinicopathological study was performed on the malignant lymphomas (MLs) occurring in HTLV-I nonendemic Japanese area with the use of REAL classification. The study group consisted of 502 patients with ML, experienced at our institution during a recent 12-year period between 1984 and 1995.

The following characteristics were noted: 1) The incidence of Hodgkin's disease is extremely low (1%). 2) Among NHL, extranodal manifestation is high (60%). 3) The cell lineages consist of B-TANK (84.1%), showing a high occurrence of the TANK-cell type (23%). 4) The B/TNK ratio largely depends on the site of manifestation, suggesting a tissue-specificity. The frequency of TANK-cell type is highest on cases occurring in skin and thymus, followed by the nasal cavity and nasopharynx (44%). 5) Diffuse large B-cell L is predominant (46%), constituting around a half. 6) Follicle center Ls. occur with a low incidence (15%); with cases bearing (14:15) accounting for only 25%. 7) Marginal zone B-cell Ls. account for 8% of the total number of NHL and 32% of gastric NHL. 8) Peripheral T-cell L of the unspecified type is common (13%), accounting for a majority of the TANK-cell NHL. 9) As to peripheral T-cell L of specific types, angiomatoelastic L constitutes 4% and angiocentric L constitutes 2%. 10) The incidence of adult T-cell L is low, being the same as in Japanese HTLV-I nonendemic area is equivalent to that among European and American patients (1%).

The following characteristics were noted concerning the prognosis: 1) Among small B-cell L, mantle cell L shows a poorer prognosis. 2) Among the peripheral T-cell L, angiocentric L., which is associated with an acute outcome, was shown to be particularly aggressive.

Patho-epidemiological characters of MLs occurring in HTLV-I nonendemic areas will be discussed in terms of REAL classification.

ARE ALL PREVIOUS MALIGNANT HISTIOCYTOSES IN FACT KI-1 POSITIVE LARGE CELL ANAPLASTIC LYMPHOMA? A NEW PERSPECTIVE?
I.M. Rossoff-Josef 1, R.M. Egerle 2, J. den Hollander 3
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Introduction: One of the most confusing aspects in the field of malignant histiocytic disorders concerns the distinction between large-cell anaplastic lymphoma (LCAL) from a tumor of histiocytic origin, malignant histiocytosis (MH). A tumor with the hematotax and eosin (H&E) morphology of LCAL that is CD30 + is almost always lymphoid in origin. On the other hand tumors that are lysozyme positive are likely to be histiocytic.

Our findings indicate the role of adhesion molecules ICAM-1 and LFA-1 in the pilomatricoma leading to mechanical obstruction by tumoral cells, hyperkeratosis and then cyst formation. It remains to determine if ICAM-1 expression is the cause or the consequence of pilomatricoma.

Clinical data: Characteristically all children presented with more or less signs of systemic illness, in addition to lymphadenopathy and hepatosplenomegaly.

Histological data: A review of the histological material was made according to an experimental scoring system as proposed by the Histocyte Society.

Conclusion: In the last eight years we diagnosed 7 patients with LCAL. Three patients diagnosed before 1992 initially as a MH, were reclassified as LCAL, as at that time immunoperoxidase antibody stains became available. So this patient group reveals no case with a true Malignant Histiocytosis. The literature (Egerle 1996, Sonneveld 1990), however, describes a fatal syndrome which seems to be a rare clinical entity, characterized by a monoclonal malignant proliferation of histiocytes. This diagnosis needs an extensive diagnostic approach concerning the histological, immunophenotypic cytogenetic and molecular biological features.
AGGRESSIVE NK CELL-LEUKAEMIA AFTER SINONASAL LYMPHOMA.


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Sino nasal non-Hodgkin's lymphomas (NHL) occur more frequently in Oriental than in Western populations. Dissemination is rare and usually involves skin and lung. A T-cell or natural killer (NK)-cell phenotype is common. A strong association with EBV has been reported.

Aggressive NK cell leukaemia-lymphoma (ANKL/L) is a rare cliniopathological entity described among Asians and occurring as a "de novo" leukaemia.

We report two unusual cases of ANKL/L developing in Caucasian females in the evolution of sinonasal NHL.

Both cases display typical features of stage IE high-grade angiocentric NHL. The phenotype of the sinonasal tumor is CD20+ CD3− DR+ CD45Ra− CD56+. EBER ARN are demonstrated in situ hybridization. After failure of an anthracylin- containing regimen, local remission is achieved by high-dose chemotherapy with stem cells support and/or radiotherapy. In both cases, systemic symptoms occur during local irradiation: fever, hepatomegaly with jaundice and lactacidosis, pancytopenia, CNS involvement, high LDH levels. Bone marrow examination shows 30-50% lymphoblastic cells, with azurophilic granulations in 1 case. NK phenotype (CD2+ CD3− DR+ CD56− 7% and DR+) and EBV+.

In these two cases, ANKL/L appears as the clonal evolution of sinonasal T-NK NHL. The etiopathogenic role of EBV and/or an occult immunodeficiency is suggested. Optimal treatment of sinonasal NHL remains to be defined.

PERIPHERAL T CELL LYMPHOMA OF ALD-I-LIKE: IS THE ORIGIN OF THE TUMOR CELLS AN IMMATURE PRECURSOR CELL IN THE THYMUS?


Aging immunologic lymphomadenopathy with dysproteinemia (AILD) is a malignant disorder clinically characterized by fever, sweats, weight loss, anemia, hepatosplenomegaly and marked lymphadenopathy. The putative tumor cells in AILD have the phenotype of mature T lymphocytes; in group 1, CD4-positive helper/inducer cells are thought to be the malignant proliferating population; in group 2, proliferating CD8-positive suppressor cells are supposed to represent the tumor cells. This is assumed, because in the different groups the CD4 and CD8 positive cells, respectively, represent the predominantly proliferating cell populations.

In cytogenetic studies, malignancy and clonality in AILD has been confirmed by the finding of trisomy 3 in about 35% of analyzed cases. Our new technique of combined immunophenotyping and interphase cytogenetic analysis (FICTION) enabled us to investigate, whether the trisomy 3 - as would be expected - is exclusively restricted to one subpopulation of T lymphocytes (CD4 or CD8). Using this approach we surprisingly found that within one case of AILD CD4 positive as well as CD8 positive T lymphocytes showed the same clonal chromosome aberration, the trisomy 3. This finding indicates that in this case of AILD a precursor T cell, not yet committed to either helper or suppressor function, possibly had acquired the trisomy 3 and then gave rise to both CD4 positive and CD8 positive aberrant T cells. Our results show that in spite of the mature phenotype of the tumor cells, the onset of peripheral T cell lymphoma of AILD-like type might be in the thymus.

LONGEVITY OUTCOME IN T-CELL LYMPHOMA

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Ninety T-cell-lymphoma (TCL) treated since 1980 have been evaluated. In 83% histology was confirmed by a German lymph node reference center. According to the Kiel- and R.E.A.L.-classification for TCL three different groups were formed: 1. large cell (incl. immunoblastic and anaplastic subtype), n = 31; 2. angioimmunoblastic (AILD = LgmXX), n = 25; 3. small cell (incl. Mycosis fungoides, Lennert- and T-Zone-Lymphoma), n = 34. Median age was 57 years (yr). Cutaneous infiltration was the leading extranodal involvement (30%).

17 patients (pat.) showed localized (IA: n = 11; IIA: n = 6) and 73 advanced disease (IIB n = 5, IIIA n = 9, IIIB n = 7, IV A n = 15, IV B n = 33). Treatment for the large cell group and AILD included polychemo (PCT) and radiotherapy (RT). Younger pat. received intensified treatment according to the BMFT-T-ALL-protocol, followed by high dose treatment and stemcell transplantation. The small-cell TCL group however was treated heterogeneously: steroids, alpha-interferon (IFN), PUVA, Pentostatin and PCT as well. 10 yr. overall survival probability (OS) was 48% for large cell TCL, 17% for AILD and 34% for the small cell entitites. Regardless of subtype, localized stages showed 10 yr. OS of 64%, advanced stages of large cell TCL 41%, AILD and all others 14%. Remarkably high were second neoplasias in 6% within a few years after diagnosis or even simultaneously. Large cell TCL appear to be successfully treated with combined modality therapy, while improvement of strategies for small cell T subtypes is required.

NATURAL KILLER CELL LYMPHOMAS: CLINICAL, HISTOLOGICAL, PHENOTYPIC AND GENOTYPIC CHARACTERISTICS


NK cells are lymphocytes that mediate cytotoxicity without prior sensitization. NK cells have phenotypic and genotypic characteristics that they express. The NK-related antigen (CD56) T cell markers such as CD2 and CD7 but do not express CD3 and T cell receptor (TCR), and their TCR locus is not rearranged. We present herein a series of 20 lymphomas presenting similar phenotypic and genotypic characteristics. Two groups of patients were observed according to clinical presentation: those with a sinonasal lymphoma (15 cases) and those with non nodal (non nasal lymphoma (5 cases), which frequently involved liver (5/5), skin (3/5) and spleen (2/5). Histology was pleomorphic and medium large cell in all cases, and angionvasin was observed in 6 cases. Phenotype was characterized by the expression of T cell markers such as CD3, CD4, CD8, CD19, and CD20, but neither CD56 and the TCR a/b and yb protein (in 20/20). Lymphoma cells were CD4/CD8- in most cases (13/15), and expressed no B cell marker (CD20, CD19 or CD22) as well as CD7 and CD69. Interestingly, lymphoma cells expressed the CD8 chain, detected by an anti-gamma antibody on paraflin sections (4/17), and expressed CD5 and CD71 but not the TCR a/b and yb protein. These findings are supported by the expression of CD71 and CD8, which are both expressed in a small percentage of normal NK cells (7/100), and some cases of sinusoidal NK cell lymphomas were associated with Epstein-Barr virus (EBV), as shown by LMP (15/15) and EBER expression (15/15). By contrast, the four extranodal/extranodal tested cases were EBV-negative. This study indicates that NK cell lymphomas do exist, and comprise 1) nasal lymphomas, which constitute a distinct EBV-associated entity, 2) systemic cases which are EBV-negative and more heterogeneous in clinical presentation.
4. Pediatric lymphoma

CYTOMORPHOLOGY OF CHILDHOOD NON-HODGKIN'S LYMPHOMAS

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Lymph nodes cytology of 72 children with newly diagnosed and
histologically proved non-Hodgkin's lymphomas (NHL) were
examined between 1984 and 1993. The patients ranged in age from 1
to 14 years (mean, 7.5 years) with a male/female ratio of 1:1.9.

Besides the qualitative characteristics of cells, the quantitative
morphometric parameters and mitotic index have been evaluated as
well.

According to the International Classification of the
Working Formulation criteria (1982) the following types of NHL have
been diagnosed: 44 (51.1%) cases of small non-cleaved cell
lymphomas (Burkitt's and non-Burkitt's variants), 15 (20.8%)
lymphoblastic, 11 (13.5%) diffuse large-cell lymphomas (large non-
cleaved, immunoblastic, large-anaplastic-Ki-1) and 2 (2.6%) mixed
cell histology NHL, arisen from small and large cells.

Our research showed that 66 from 72 children (91.7%) had highly
malignant blastic types of NHL which means their absolute
predominance among childhood NHL. Furthermore morphometric
analyses allowed to establish the objective criteria to identify two main
types of childhood NHL. We found out that small non-cleaved cell
lymphomas and lymphoblastic NHL significantly differ in size of cell
area and perimeter as well as that of the nucleus (P<0.001).

These results suggest that it is possible to distinguish different
types of childhood NHL using only cytology.

EXPRESSION OF RAS, MYC, LMP AND PCNA IN CHILDHOOD
LYMPHOMAS

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Haematology Oncology, Pathology, University of Crete Medical School,
University Hospital of Heraklion, 1352 Crete.

Paraffin sections from 21 cases of Hodgkin's Disease (HD), 23 cases of non-
Hodgkin lymphomas (NHL) and 34 cases of reactive lymphadenitides
occurring in childhood, were examined for the expression of ras, myc, PCNA
and LMP, using the method of immunostaining. Ras-p21 protein was hardly
expressed in all cases that were examined. Myc-p62 protein was frequently
detected in all cases of lymphomas and mainly in HD. LMP was found in
12/21 (57%) cases of HD, but in none of the NHL cases and of the reactive
lymphadenitides that were studied. LMP reactivity was restricted to
Hodgkin's and Reed - Sternberg cells. All cases of HD and NHL showed
PCNA reactivity, with a considerable variation of proliferation rate among
different subtypes of both groups. The above results suggest that myc
oncogene rather than ras is implicated in the pathogenesis of lymphomas.
In more than half of the HD cases studied Epstein Barr virus seemed to be
involved, while the PCNA index could be helpful in all childhood lymphomas
to assess their proliferating capacity.

SERUM LACTATE DEHYDROGENASE ISOENZYME IN PEDIATRIC
LYMPHOMA, POSSIBLE ROLE IN DIAGNOSIS AND TREATMENT
MONITORING.

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Cancer Institute & Ein Shams Hospitals, Egypt.

The pretreatment levels of serum total lactate dehydrogenase (LDH)
and its isoenzymes as well as beta 2 microglobulin (B2M), were
measured in 50 cases with HD and NHL, pediatric cases and
compared with 10 matched healthy controls. 1/4 of cases only
showed the first 3 bands and other cases showed the first 4 bands
of LDH. Among the HD cases, the LDH1, LDH2, LDH3 and LDH5
values were significantly raised as compared to the controls (240%,
225%, 294%,1 95% and 246%). LDH2 and LDH5 were significantly
increased (235% and 413%). It was noticed that the LDH2 was most
specific in cases of NHL. On the other hand, the LDH5 was most
sensitive in the HD cases. After treatment, LDH was significantly
decreased in 60% of NHL cases as compared to 71.43% decrease
among the HD cases.

Among the NHL responders, the LDH and the LDH-isoenzymes
were as follows: LDH1:126%; LDH1:117%; LDH2:165%;
LDH3:142.9%; LDH4:86.9% and LDH5:202%. On the other
hand, among the HD responders, the LDH was 122%, while the
isoenzymes levels were: LDH1:172%; LDH2:103%; LDH3:96.8%;
LDH4:75.9% and the LDH5:288%. Serum level of 62M was elevated in
all untreated cases while in 70% of treated cases it was decreased.
Thus LDH isoenzyme can be used to differentiate between NHL and
HD and in follow-up.

N-RAS AND K-RAS MUTATIONS IN CHILDHOOD LYMPHOMAS

A. Sakalidou, D. A. Spandidos, M. Kofa, T. Liloglou, P. Kanavaros,
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National Hellenic Research Foundation, Athens.

N-ras and K-ras mutations have been implicated in several haematopoetic
malignancies. However, there are limited available data regarding ras
mutations in childhood Hodgkin's and non-Hodgkin lymphomas. We applied
the polymerase chain reaction technique (PCR) combined with RFLP analysis
in order to investigate the presence or not of codon 12 point mutation of N-ras
in 40 cases of childhood lymphomas. From these cases (mean age 9 years old)
23 were Hodgkin's Disease (2 Lymphocyte Predominance, 2 Lymphocyte
Depletion, 10 Nodular Sclerosis and 9 Mixed Cellularity) and 17 were non-
Hodgkin lymphomas (12 T-cell lymphoblastic lymphomas and 5 B-cell
lymphomas). We found point mutation in only one case of T-lymphoblastic
non-Hodgkin lymphoma. We also investigated the presence of K-ras mutation
at codon 12, in 25 of the above cases (15 cases of Hodgkin's Disease and 10
cases of non-Hodgkin lymphomas), but none was found. These findings
suggest that ras genes do not seem to play a key role in the development of
childhood lymphomas.

4. Pediatric lymphoma
IL-1 PRODUCTION DURING THE COURSE OF NON HODGKIN'S LYMPHOMA (NHL) AND HODGKIN'S DISEASE (HD) IN CHILDREN

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Total number of 74 children with biopsy proved NHL and HD, 47 boys and 27 girls, aged from 0.5 to 15 years were included to the study. IL-1 production was detected according to the method based on inhibition of autologous erythrocyte rosette formation by thymocytes of CBA mouse. Thirty seven healthy children served as the control group. It was observed that IL-1 production in children with NHL and HD before beginning of therapy was lower than that obtained in control group of healthy children.

<table>
<thead>
<tr>
<th>IL-1 production (median values)</th>
<th>NHL</th>
<th>HD</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>before therapy</td>
<td>2.2</td>
<td>9.6</td>
<td>15</td>
</tr>
<tr>
<td>during chemotherapy</td>
<td>4.75</td>
<td>6.2</td>
<td>15</td>
</tr>
<tr>
<td>after chemotherapy</td>
<td>9.3</td>
<td>5.1</td>
<td>15</td>
</tr>
</tbody>
</table>

The IL-1 production decreased after starting chemotherapy of HD (m=-4.75u), while relative increase of IL-1 was observed in patients with NHL (m=-6.2u). It was found that in children with HD and NHL during the whole therapy the IL-1 production, was significantly lower than that observed in the control group of healthy children (p=0.005). During 10 years period after the end of therapy in both group of children (NHL and HD), IL-1 median values didn't reach values of the control group. On the basis of our results and literature data we can conclude that IL-1 production could play essential role in pathogenesis and clinical course of childhood NHL and HD.

INITIAL MANAGEMENT OF BURKITT'S LYMPHOMA IN CHILDREN: IS THERE STILL A PLACE FOR SURGERY?

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Purpose - This retrospective study compared the overall survival, the event-free survival, and the timing of chemotherapy in patients with advanced Burkitt Lymphoma with and without laparotomy.

Material And Methods - Thirty five patients with advanced abdominal Burkitt Lymphoma treated at least partially at the Centre Léon Bérard between 1981 and 1992 were included in this study. The diagnosis was obtained by laparotomy (LAP group) in 21 patients (17 stage III, 4 stage IV) and by other methods (non-LAP group) in 14 patients (5 stage III, 9 stage IV).

Results - The overall survival (71% and 93%) and the event-free survival (66% and 79%) were similar in the LAP and non-LAP groups, while the relapse rate was 5 (3 local) in the LAP group compared to 3 (none local) in the non-LAP group. The local complication rate (9/21 versus 2/14) and the toxic deaths rate (2/21 versus 1/14) were slightly higher in the LAP group. Laparotomy also caused delays in therapy and increased the overall hospital stay. The median interval from diagnosis to the start of the fourth course of chemotherapy was 57 days compared to 48 days and the average hospital stay was 44.4 days compared to 39 days for the LAP and non-LAP groups respectively.

Conclusion - Since advanced Burkitt lymphoma can be diagnosed by fine needle aspiration, and chemotherapy cures more than 80% of the patients, there is no need for initial surgery, apart from acute emergencies. Furthermore, laparotomy delays chemotherapy and might reduce the survival rate.

PECULIARITIES OF THE APPROACH TO NON-HODGKIN'S LYMPHOMA (NHL) THERAPY IN CHILDREN WITH UNFAVORABLE PROGNOSTIC FACTORS

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Therapy of childhood NHL was performed individually according to a prognostic factor. The unfavorable prognostic factors include age above 12, boys, mediastinal involvement more than 5 cm, initial involvement of bone marrow and retropertioneal lymphatic nodes. Twenty-three NHL patients with the unfavorable prognostic factors were under the observation. Eighteen of them had Stage III and five children had Stage IV NHL. The children were treated by a three-step program including induction, consolidation and re-induction therapy. At the initial stage of the tumor process, i.e. at the induction stage, the treatment was intensive and individual. In the bone marrow involvement the patients were treated with the same drug combination as it was used in acute lymphoblastic leukemia: vincristine, L-asparaginase, Adriamycin+prednisolone, methotrexate, cytosine arabinoside. At the stage of consolidation the patients received cytosine arabinoside, 6-mercaptopurine, methotrexate in high doses with leucovorin, vepezid. The complete clinical and hematologic remission has been achieved in 70% Stage IV patients and in 85.5% Stage III patients. Thus, therapy of childhood NHL with unfavorable prognostic factors at the initial stage of the tumor process must be individual and intensive aimed to eradicate the tumor process.
CHILDHOOD PERIPHERAL T-CELL NON HODGKIN'S LYMPHOMA (PTL): REPORT OF TWO CASES.

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T-NHL are relatively more common in children than in adults. The majority of these are lymphoblastic NHL, whereas post-thymic NHL are very unusual. In our series CD30+ anaplastic large cell NHL (ALC) represented 10% of all NHL (Ann Oncol 6:915-20,1995); other subtypes were exceptionally rare. We here report on two children with PTL other than ALC observed in our Institution over the last ten years. Both pts (one male and one female, aged 9 and 5 yrs, respectively) presented with a long-lasting history of multiple lymphadenopathies, liver and spleen enlargement, slight nonpruritic pruritis, leukosferesis, lymphopenia, elevation of alkaline phosphatase, poor height and weight gain. Both children were HIV-negative. In one case mediastinal enlargement and pleural effusion were detected too. Bone marrow biopsies and smears, as well as spinal fluid cytology were always negative for malignant cells. Both children were submitted to subsequent inconclusive multiple lymph node biopsies with histological diagnosis of prominent reactive hyperplasia involving follicles, with plasmacytosis of the medullary cords, and/or paracortical areas. PTL were diagnosed on the fifth and fourth biopsy, respectively. Subtypes according to the updated Kiel classification were angioimmunoblastic and T-zone PTL. Malignant lymphoid cells revealed CD2+, CD3+, CD4+, CD5-, CD7+, TdT-, CD8-, CD30- phenotype. PCR analysis for EBV-DNA was performed on all biopsy specimens and was negative. Both children were submitted to polychemotherapy according to the protocol adopted for lymphoblastic NHL when complete remission was obtained after the induction phase, bone marrow transplantation (BMT) was proposed. The boy's family refused BMT and the girl died 14 months later for progressive disease. The girl was transplanted in December 1995 and was alive in CR at the time of this report. Our data confirm the rarity and the difficult diagnosis of PTL in children and its dismal prognosis with conventional chemotherapy.

BRIEF INTENSIVE CHEMOTHERAPY FOR CHILDREN WITH HIGH-RISK SMALL NON-CLEAVED CELL LYMPHOMA (SNCC) AND ACUTE B-CELL LEUKEMIA (B-ALL).

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Since November, 1989, 17 children newly-diagnosed with high-risk SNCC (stages III with LDH >500 U/L or stage IV/B-ALL) have been treated with a CNS-based chemotherapy: mephalan, cyclophosphamide, thiopeta, adriamycin, etoposide, vincristine, high-dose methotrexate (HD-MTX), dexamethasone plus intrathecal (IT) MTX and cytosine arabinoside (ara-C). Consolidation Phases: HD-ara-C, L-Asparaginase, etoposide, HD-MTX and IT MTX and ara-C. Reduction and Reconsolidation Phases: essentially repeat once of both Induction and Consolidation at somewhat less intensity. Only the first 3 pts. received Reconsolidation. Duration of therapy as planned was 3 months (mos.). No irradiation or stem cell transplant were permitted. 17 pts at diagnosis were stage IV (12 B-ALL and 6 with CNS involvement) and 10 pts. stage III. Of the 27 pts., 26 achieved a complete remission (CR) with 1 toxic death in induction. One stage III pt. experienced local abdominal relapse at 4 mos. 2 pts. with B-ALL experienced early marrow relapse after brief CR. No pt. with CNS involvement has relapsed. Four pts. received induction therapy, discontinuing after therapy-related pannynphalopathy (1), tumor-related myelopathy (1) and severe fungal sepsis (2). Two additional pts. received induction therapy followed by 1 and 3 cycles of "CHOP" therapy, due to severe toxicity in Induction; all these toxicities occurred in the early phase of the program, prior to incorporation of G-CSF and decrease in duration of steroids in Induction per protocol. 22 of 27 pts. (81.5%) remain free of disease without recurrence 6 to 74 mos. (median 51 mos.) from diagnosis. The initial toxicities of this regimen have been markedly reduced since incorporation of G-CSF and reduction of duration of dexamethasone use in Induction. This regimen appears highly effective for high-risk SNCC pts., including those with CNS involvement.

CLINICOPATHOLOGICAL FEATURES OF CHILDREN WITH Ki-1-POSITIVE ANAPLASTIC LARGE-CELL LYMPHOMA. CENTRAL NERVOUS SYSTEM INVOLVEMENT IN TWO CASES.

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Ki-1-positive anaplastic large-cell lymphomas [ALCL] have some particular clinical features. While extranodal localizations are common, initial CNS involvement has been virtually not reported and meningeval recurrence is also extremely rare. Here we report the clinicopathological features of 9 patients with ALCL, treated at the Emma Children's Hospital from 1990 to 1995, of whom two had CNS disease. Characteristics of patients (n=9): age,1.5 to 13 year (median 9 year); 5 M/4 F; stage I n=1, II n=3, III n=3, IV n=2; sites of disease at presentation were: lymph nodes in 8, skin in 2, CNS, bone, lung, liver, spleen in 1-1 pts resp.; no bone marrow infiltration has been found. Tumor cells were of T-cell origin in all cases. From six patients where cytogenetical study succeeded three had translocation t(2;5)(p23;q35). Seven patients have been treated uniformly with an intensive chemothera- pion of one year. One patient (initially II lymphnode sites) experienced a recurrence 15 month after dg with multiple lymphnodes and extranodal (CNS, skin, lung, skin) sites. She died of infection in 2nd CR 26 month after the dg. All the other patients are in 1st CR with a follow-up time of 2 to 62 months (mean: 26 mo). Both children with CNS involvement, one at the diagnosis and one at the recurrence, had at that moment an advanced disease with multiple nodal and extranodal localizations. CONCLUSION: Our results are consistent with the data on the specific clinical presentation of the disease and indicate that CNS involvement can occur in children with ALCL even in patients with an initially localized disease. Treatment results are satisfying.

EVALUATION OF LONG TERM THERAPY RESULTS OF ADVANCED NHL-B IN CHILDREN TREATED WITH THE COAMP PROTOCOL. REPORT BY POLISH PEDIATRIC LEUKEMIA Lymphoma STUDY GROUP (PPLLSG).


During the years 1983 - 1987, 40 children with NHL, stage III and IV, aged 10 months to 15 years were treated according to the COAMP. Histopathological classification was done according to the Kiel scheme. The staging system of S. Murphy was used for prognostic stratification. The goal of this study was to evaluate the long term therapy results of the protocol. Thirty (75%) of 40 pts presented in stage III, 10 (25%) in stage IV. (8 with the BM invasion and 2 with the CNS involvement). The most common localization of the primary tumor was the abdomen 23, (57.5%) children of which 19 had the huge tumor mass. Thirty eight (95%) children achieved complete remission (CR). Two children did not respond to treatment. Two patients died in CR due to infection. Relapses occurred in 12 of 19 patients in stage III and IV with huge abdominal mass. As of date, following 15 to 147 months observation (median 101 months) 27 patients are alive. The probability of survival of all children in the analyzed material after 147 months of observation is 67.5%. The probability of EFS for children in stage III is 72.4% and in stage IV 33.0%.

Because of the satisfying results in B-NHL stage III COAMP protocol should be considered in the situation, when more intensive therapy cannot be applied (i.e. chronic B or C hepatitis).
B-NHL IN CHILDREN TREATED ACCORDING TO B-NHL BFM-90 PROTOCOL.

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From January 1991 to January 1996, 21 children were admitted and treated in our center. Diagnostic procedure included lymphnode biopsy, determination of LDH level, clinical examination and cytogenetic study. Morphological examination and cytochemical analyses of extirpated lymphnode were performed by single pathologist. Regular immunophenotyping was not performed due to deficiency of monoclonal antibodies. Among our group of patients majority were boys (20) and only 1 girl. At presentation they were diagnosed as stage II (7), stage III (6), stage IV (8). In all of them we applied B-NHL BFM 90 protocol with initial good response. In 4 patients local recurrence and further progression of the disease were registered during first 2 months of treatment. Two patients remitted immediately after the cessation of therapy. Fourteen patients are in complete remission, 7 patients died, 1 from septic complications and others because of progression or recurrence of the underlying disease. During application of B-NHL BFM 90 protocol we used: rG-CSF-Neupogen ROCH which made possible to follow strictly the schedule of the protocol.
CHILDHOOD HODGKIN’S DISEASE IN CASABLANCA MOROCCO

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The purpose of this retrospective study is to give an overview of childhood Hodgkin’s disease (CHD) i.e. aged 15 years or younger, treated in Casablanca. All cases of CHD proven by biopsy seen between 1980 and 1994 are included. Staging procedure included clinical history, physical examination, chest x-ray, ultrasound of abdomen, lymphangiography or CT scan when possible, and bone marrow biopsy. Exploratory laparotomy is not used. From 1980 to 1981 patients are treated according to MOPP combination and since then MOPP/ABV or ABV combination is used. Extended field radiotherapy is used from 1980 to 1988 and involved field afterward. One hundred eighty five cases of CHD are referred to our institution. The male/female ratio is 3.3. Thirty four patients (18.3%) are 5 years old or younger. Fifty four percent of the patients presented with mediastinal mass and 57.2% presented B symptoms. Advanced stages III and IV are found in 29.7% and 31.8% respectively. Histologic subtype when mentioned is 1 in 25 cases (19.4%), 2 in 33 cases (27.1%), 3 in 64 cases (49.6%) and 4 in 5 cases (3.9%). Hundred and six patients are eligible for treatment evaluation. In these patients, complete remission is obtained in 89%. Overall survival at 5 years is 92% and disease free survival 70%.

CHILDREN OF PARENTS WITH HODGKIN’S DISEASE.

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The data are presented on the course of pregnancy, delivery and subsequent development of 33 children born to parents treated for Hodgkin’s Disease. Thirteen women in the clinical stage II and III were delivered of 29 infants (16 daughters and 13 sons) and three men (IIA and IIIA) had four daughters. The parents were treated by a combination of irradiation and chemotherapy (CDOPP/ABV) except one case treated with irradiation only and two cases by chemotherapy. The gestation period, parameters of infants at delivery and the subsequent physical and mental development are normal. In one instance (a girl, now twelve and a half years old) the child was born with malformations of the extremities: according to the geneticist this was not related to the previous treatment of the mother. The second child (a son) of this mother has been normal. Four children are over 12 years old. The autors are of the opinion and apply it in the therapeutic protocol in patients of fertile age and do not irradiate nodes in the pelvic region. In treated patients they allow pregnancy only after three or preferably five years following remission.

RESULTS OF TREATING CHILDREN SUFFERING FROM HODGKIN LYMPHOMA IN CROATIA

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On the Hematology-Oncology Department Children’s Clinic Salata, Zagreb, which is Republic Centre for children with malignant diseases in Croatia, 103 children were treated for Hodgkin lymphoma from January 1974 till December 1995. Exclusively with irradiation were treated 8 children who were in the first or second stage of the disease; seven of them are still alive, while one died during relapse of the disease. 46 children were treated with Protocols MOPP and ABVD combined with irradiation; 36 (87%) of these children are still alive and show no sign of the disease. In 10 (22%) cases there were relapses during which 6 (13%) of the children died. 35 children who were treated with Protocol HD-85 are still alive; 32 of them are still in the first remission. 14 children were treated with Protocol DAL-HD 90 and all of them are so far in the first remission. Of the total of 103 children 25 were still alive on the 31st December 1995 (25%); 89 (86%) of the children are still in the first remission. The achieved results are satisfactory and do not differ significantly from those achieved at similar Centres in Europe.

TREATMENT RESULTS OF 210 CHILDREN WITH HODGKIN’S DISEASE IN A SINGLE INSTITUTION

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From June 1984 to December 1992, 210 previously untreated children (157 male and 53 female) with Hodgkin’s disease were treated and followed-up in a single institution. The median age was 8 years ranged between 1-18 years and 17.6% of cases were less than 5-years of age. The stage distributions by clinical staging were stage I in 82, II in 50, III in 58 and IV in 20 patients. Most of the patients (81.5%) showed mixed cellularity type of histology. Patients with stage I and II disease were treated by three cycles of CDPP plus low-dose involved field radiotherapy. Stage III and IV disease were treated by six cycles COPP plus low-dose involved field radiotherapy. The over all and event-free survival rates were 94.7 and 81.5% at 5 years, 94.7 and 79.6% at 8 years for clinical stage I-II; 65.6 and 54.8% at 5 years, 74.6 and 54.8% at 10 years for stage III-IV, respectively. These results showed that three or six cycles of chemotherapy, depending upon the stages, combined with low-dose radiation involved-field therapy will give high survival rates.
A STUDY OF SURVIVAL IN PEDIATRIC HODGKIN'S DISEASE
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During the period 1 January 1984 - 31 December 1983, 73 previously untreated children with a histologic diagnosis of Hodgkin's disease were evaluated and treated at Oncological Institute Cluj-Napoca.

The children were 3-16 years old: 43 boys and 30 girls.

We have treated 8 children with pathologic stage I, 37 with stage II, 15 with stage III and 17 with stage IV. The children were treated with chemotherapy alone (COPP, ABVD) or with chemotherapy + radiotherapy.

Nodular sclerosis was the most common histologic type: 26 patients NS grade I and 10 patients NS grade II. The mixed cellularity type was diagnosed at 22 patients, lymphocyte predominant type at 4 patients and lymphocyte depletion at 4 patients.

The survival rate of children at all stages was 74.33% at 5 years, at stage I was 100%, at stage II 91.3%, at stage III 51.85%, at stage III 66.87% at Stage IV 45.80%.

We have determined the relationship of histopathologic type to survival. At 5 years 100% children with lymphocyte predominant type and 77.14% with mixed cellularity type.

THE PATTERNS OF MALIGNANT LYMPHOMAS IN TURKISH CHILDREN
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Malignant lymphomas (ML) constitute the 2nd most common malignancies among childhood neoplasms in Turkey. During the period of 1964 to 1994, 262 patients with ML (lymphoma (ML) were diagnosed at our center, 180 being Hodgkin's Lymphoma (HL) and 85 Burkitt's Lymphoma (BL). The HL represented nearly 50% of total ML and BL was 46.7% of NHL. Clinico-epidemiological and histopathological findings of 175 HL and 65 BL cases have been analysed retrospectively. HD was characterized with the striking features of Type I Pattern, a developing country pattern with high frequency of mixed cellular subtype (60.6%) in predominantly male children (76%) during the first decade of their life (75%) observed over the years. Majority of the ML patients were from low and low-middle SES groups. Presenting findings of BL were between African and American types with 44% maxillo-facial tumors (mainly jaw and orbit) at diagnosis. Serologic study revealed elevated lgG antibodies to EBV-VCA in ML patients (93.4% in HL and 90% in BL respectively) as compared to 311 healthy children (77.1%). GMT of anti-VCA were 1/148 for HD, 1/312 for BL, and 1/193 in normal children respectively.

EBV-DNA was studied in 55 ML by PCR and immunohistochemical (IHC) methods. EBV-LMP was detected in 62.5% of 40 HL patients by IHC and the presence of EBV-DNA was shown both in HL (88%) and BL (95.4%). Blood and hair zinc (Zn) and Selenium (Se) levels were found to be decreased at admission. Altered cellular immunity was also detected in several parameters of T cells, namely anergy, diminished T cell counts and CD4 cells and decreased response of lymphocytes to PHA, EBV in the presence of secondary/constitutional immune deficiency due to low SES may play a role in ML of Turkish children. In conclusion, Type I epidemiologic pattern of HD was found to exist among Turkish children over 30 years associated with low SES, EBV, trace elements deficiency and immune alteration. Furthermore an "intermediate type of BL" seems to be prevalent in Turkey associated with high frequency of EBV, and low median age (5 years).

EVALUATION OF TWO EBV PROTOCOLS IN CHILDREN WITH HODGKIN'S DISEASE.
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We used two different (E) doses (group 1 = 30 mg/m², group 2 = 0.25 mg/m²) under EBV protocols: bleomycin 10 mg/m², vinblastin 6 mg/m², days 1 and 15, and 15, number of cycles (cyc) was: CS I-II 4, CS III I-II 6, CS III I-II 6, CS III I-IV 8, and XRT 3000 Cgy to involved field. Group 1 included 24 children (mean age 8,5 years, range 2-14), 17 male, mean follow-up 60 months, treated between 1987 and 1990. Histology were NS 42%, MC 50%, LP and I.I each 4%. Prognostic factors (FY) were CS III 45%, B symptoms 29%, MM >10% 12.5%, bulky disease 5 cm (BD) 58%, K 70% 12.5%. Group 2 included 20 children (mean age 9.2 years, range 4-16), 14 male, mean follow-up of 24 months, treated between 1991 and 1993. Histology were NS and MC each 45%, LP and I.I each 5%. RE were CS III 40%, B symptoms 30%, MM >40% 20%, BD 50%, K <70% in 10%, high risk pts (FY >3) for groups 1 and 2 were 25% and 54%, respectively. Differences in survival and in complete remission (CR) were not significant at 24 and 36 months.

Comparison between high and low risk pts from both groups were not significantly different in OS, DFS, and CR in DFS. Conclusion increasing E from 30 mg/m² to 60 mg/m² under EBV regimen did not provide additional improvement in survival or CR in children with Hodgkin's Disease.

THE PATTERN OF CHILDHOOD HODGKIN'S DISEASE (HD) WITH KVPV CHIMIOTHERAPY AND RADIOTHERAPY (IP-R): EFFECT OF DOSE AND SCHEDULE MODIFICATION ON TREATMENT OUTCOME.
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From 1970 to 1987, 86 children with HD I (1-IV) were undergoing initial treatment with MVPP (methotrexate/methylprednisolone/CCNU/polychloroimidazine/IFP) as a combination with IP-R. Treatment modifications were frequently made according to dose and schedule. The impact of the treatment on the progression-free survival (PFS) and the overall survival (OS) in the population is that the three drug M (V, N, and P) delivery (EVM) were calculated as described by Canu et al. J. Clin. Oncol. 1981, 3, 146.

The progression of disease (PD) during the initial treatment in our center was the most common feature. The rate of macroscopic disease delivery during the 10-year observation period was 5 cycles of MVPP on schedule (P = 0.05) for children with D34 (0.6) and 0.01 for children with D134 (0.6). The difference was significant, and plateau was obtained after 14 and 8 months with the probability of 0.03 and 0.05, respectively.

Relapse (R) were found in 13 cases, most often in stage I/II-IV (52%) and unexpectedly numerous in stage IA (16%). For 31 children in stage IA non of the three drugs had impact on R; only for 5 children in stage II-IV, the rate of R was significant (P = 0.01). The impact of vinblastine during the first three cycles (I133) had significant influence on the rate of R. For children with I133 = 0.7 and I133 = 0.7, the rate of R was 0.92 and 0.21 respectively, (P = 0.01).

The PD frequency was limited by providing more than 60% of the planned polychloroimidazine dose during the treatment period (54 days) designed for the completion of the first two MVPP cycles.

In advanced HD (III-IV), maintenance of the vinblastine dose intensity in the first three cycles above 70% of the planned essentially significant for R prevention.

The correct rate of drug delivery in the early period of HD treatment is important for prevention of both progression and relapses, especially in advanced disease.

4. Pediatric lymphoma
SERUM LEVELS OF CYTOKINES IN CHILDREN WITH HODGKIN'S DISEASE


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Impaired cell-mediated immunity in untreated Hodgkin's disease (HD) has long been recognized. The nature of the defect in HD remains enigmatic, although evidence points to immunosuppressive factors in serum or plasma of these patients. Cytokine profile, primarily serum interferon-alpha (IFN-α) were determined in 50 biopsy-proven HD patients and in 15 age matched healthy controls. A sandwich enzyme immunoassay was used for cytokine and receptor measurements. The study group consisted of 37 boys and 13 girls with an age range from 3 to 17 years (median 8 years). In all patients the diagnosis was based on histological findings, the nodular sclerosis subtype found in 6 patients, mixed cellularity in 42 patients, lymphocyte predominance in 2 patients. Of 50 patients, 8 were in stages I and II, 12 were stages III and 30 were stage IV according to Rye classification. Serum sIL-2R levels were found to be significantly high in HD patients and correlated with disease stage as compared to the remission group and normal children (p<0.01). The number of patients with detectable serum sIL-2R levels was significantly higher in patients with HD as compared to the control group. IL-4 was undetectable in all patients. TNF-α levels were found to be high in patients with advanced disease stage and 3 symptoms, whereas serum TNF-α levels in patients with early stage of disease were not significantly different from those of normal subjects. The role of these circulating cytokines in the clinical presentation of HD remains unclear. Our data indicate that serum IL-2 is detectable in a subset of patients, however, in a recent study IL-2 mRNA was found to be undetectable in HD tissues. Collectively, IL-2 detected in patients with HD could actually be produced outside of the tumor tissues and be trapped by the high levels of circulating sIL-2R in these patients. Since sIL-2R is capable of binding IL-2, it may have an immunoregulatory role by competing cellular IL-2R for the ligand and thus down-regulating the immune response. In this regard, the sIL-2R which, by binding to the host's growth factors, may inhibit the normal immune response attempting to eliminate those tumor cells. Understanding the biological significance of circulating cytokines and/or receptors will render to clinical applications of cytokine directed therapy, representing a new perspective for the treatment of certain neoplastic diseases including pediatric lymphoid malignancies.

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ENDOCRINE DISORDERS SECONDARY TO CHEMORADIOThERAPY IN PEDIATRIC MALIGNANT LYMPHOMAS

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This study was undertaken to determine the late endocrine disorders due to chemoradiotherapy in children with malignant lymphomas. We studied 17 subjects, 15 were males and 2 were females. Patients had been treated with MOPP/COPP, ABVD, CHOP combination chemotherapy and radiotherapy as involved field or mantle form. At diagnosis, median age was 7 years ranging between 3 to 11 years. Median follow-up was 15.217 (8.874-17.416) at the study. Median height SDS was -0.121 (-1.75, +1.34); median treatment period was 12 months (3-48 months). Obstructive testes were detected in four cases. The thyroid gland was palpable in 11 patients and II in one case, la in two cases and lb in three subjects, according to WHO criteria, at physical examination. Thyroid function tests were evaluated in 15 subjects. Normal thyroid functions were found in 12 patients. Subclinical hypothyroidism was present in 4 cases. Sexual maturation and gonadal function was determined in 14 subjects. Diagnostic procedures consisted of interviews concerning sexual maturation, menstrual pattern, evaluation of puberty maturation, semen analyses, and hormone measurements. Three cases were in P2, four cases in P3, four cases in P4 and three in P5 according to Tanner-Marshall criteria. Five cases showed normal gonadal function. Seven patients showed normal Leydig cell function. There were single case examples for Leydig cell failure + azoospermia and retarded puberty status + azoospermia. Sexual maturation and gonadal functions were normal in 2 female subjects.

Conclusion: The endocrine late effects should be looked for in patients who have received chemoradiotherapy and hormonal replacement should be substituted there be a need to cases with subclinical hypothyroidism.

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