Diffuse large B-cell lymphoma

Historical Overview of Aggressive B-Cell Neoplasms

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Descriptions of lymphoid neoplasms emerged in the 19th century, with Hodgkin's disease, leukemia, lymphosarcoma, and lymphoma. In the early part of the 20th century, the "reticuloendothelial system" was described consisting of the "reticulo-endothelial precursor cells" or "lymphocytes", as identified by von Hansemann, and its precursor in the 1920s. The naming of the lymphocytes reflected uncertainty concerning the nature of neoplastic cells with large size and other features that called into question their relationship to lymphocytes, which were recognized as only small, obligate end cells to lymphoid tissue, and cell structures that functioned in immune responses. Cells in germinal centers of lymph nodes were recognized as B cells proliferating in response to antigen, and the "immunoblast" was described as the pre-cursor of the lymphocyte. Lennert (1969) went on to recognize that some of these lymphocytes were not of "histiocytic" or "reticulo" origin, but instead were immunoglobulin-producing B cells. He judged that most corresponded to proliferating cells of the germinal center—initially called germine blasts, but later changed to centroblasts to avoid confusion with germ cell tumors—while some resembled immunoblasts. In 1974, the Kiel classification used the terms centroblastic and immunoblastic lymphoma; the Lukes-Collins classification used the terms "large noncleaved follicular center cell" and immunoblastic for similar tumors. Both classifications recognized Burkitt lymphoma as a B-cell neoplasm. Since then, it has been generally accepted that most tumors previously classified as "reticulo-cell sarcoma", "histiocytic lymphoma", and "undifferentiated lymphoma" are in fact neoplasms of proliferating B cells. The understanding of the morphologic, clinical spectrum of aggressive B-cell neoplasms has improved with the advent of immunophenotyping and molecular genetic studies, identifying many "undifferentiated" neoplasms as B-cell lymphomas. In the 1980s and 1990s many variants and subtypes of large B-cell lymphoma were described, including primary mediastinal (thymic) large B-cell lymphoma, T-cell/histiocyte-rich large B-cell lymphoma, intravascular large B-cell lymphoma, lymphomatoid granulomatosis-type large B-cell lymphoma, and the immunodeficiency-associated lymphomas such as primary effusion lymphoma and plasmablastic lymphoma. In addition, the distinctive features of large B-cell lymphomas in particular extranodal sites have been recognized, including CNS lymphoma and primary lymphoma of bone. Studies using the Kiel classification found that cases classified as immunoblastic appeared to have a worse prognosis than those classified as centroblastic, but these studies have been difficult to reproduce elsewhere. Thus, despite the many advances, most cases remain simply "large B-cell lymphoma" without distinctive histologic or immunophenotypic features that would explain the clinical heterogeneity of this disorder. Recently, several groups have used microarray analysis to detect changes in gene expression that could further stratify the heterogeneous group of diffuse large B-cell lymphoma. Two early reports suggest that cases with a "germinal center" pattern of gene expression had a better prognosis with current therapy, and revealed previously unrecognized genes whose expression was associated with a poor outcome. These studies may translate back into immunophenotypic and even morphologic markers that can help subdivide this group of cases in a biologically and clinically meaningful way.

Present State of Diffuse Large B-Cell Lymphomas, Including Morphological Variants

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The category of "diffuse large B-cell lymphoma" (DLBCL) in the WHO classification represents one of the commonest categories (30–40%) of non-Hodgkin's lymphoma. The neoplastic cells are typically large, with nuclei larger than those of reactive lymphocytes. In the Kiel classification, mediastinal large B-cell lymphoma, intravascular large B-cell lymphoma and primary effusion lymphoma are considered as subtypes of DLBCL with unique clinical, immunophenotypic and genotypic features. The WHO classification recognizes several morphologic variants of DLBCL such as centroblastic (with or without lymphoepithelial involvement) and centrocytic (with >20% immunoblasts), T-cell/histiocyte-rich and anaplastic (with >20% pleomorphic cells) and plasmablastic, which presents in the oral cavity in the setting of HIV infection. DLBCL with expression of full-length AKT deserves consideration as a distinct clinicopathologic entity, and has been considered as distinct from plasmablastic lymphoma. DLBCL with expression of full-length AKT often shows a unique histologic appearance mimicking plasmablastic lymphoma, while some resemble immunoblasts. Other rare DLBCL with a predominant sinusoidal growth pattern, but negative for ALK protein, may also be identified. The variable histology and cytology of DLBCL suggests significant biological heterogeneity.

Immunophenotype: Diffuse large B-cell lymphomas express pan-B markers, e.g. CD20, CD75 and CD79a, but may be "aberrant" lacking of staining with one or more of these, in particular in DLBCL expressing the full-length AKT protein and plasmablastic (CD20–, CD79a–). Surface and/or cytoplasmic immunoglobulin (usually IgM) can be demonstrated in 50% to 75% of cases. About 10% express CD5 and 30% to 50% CD10. Bcl-2 is expressed in approximately 80% of cases and is independent of bcl-6 rearrangement. Coexpression of CD10 and Bcl-6 is considered to be a marker of a diffuse large B-cell lymphoma arising from follicle centres. Approximately 10% of DLBCL, particularly those with anaplastic morphology, express CD30 and EMA. Bcl-2 protein is expressed in two thirds of cases. The proliferating fraction, as detected by Ki-67 staining, is usually high (more than 60% of neoplastic cells). A proportion of DLBCL associated with Epstein-Barr virus (LMP1+, EBER+) and are mostly observed in patients with immunodeficiencies or in DLBCL resulting from transformation of low grade lymphoma.

Cytogenetics: Most DLBCL have rearranged IgH genes and show somatic mutations. Rearrangement of the bcl-2 gene, due to the reciprocal chromosomal translocation involving the 14q32 region (bcl-2 gene), occurs in 15 to 20% of DLBCL suggesting a relationship to follicular lymphomas in these cases. About 25% of DLBCL show reciprocal chromosomal translocation involving the 3q27 region (bcl-6 gene). Bcl-6 rearrangement is more frequent (approximately one-third of cases) suggesting cryptic rearrangement in some cases. Bcl-6 and bcl-2 rearrangements are practically mutually exclusive in DLBCL. Approximately 5–7% of DLBCL express the full-length ALK protein and plasmablastic (CD20–, CD79a–). Surface and/or cytoplasmic immunoglobulin (usually IgM) can be demonstrated in 50% to 75% of cases. About 10% express CD5 and 30% to 50% CD10. Bcl-2 is expressed in approximately 80% of cases and is independent of bcl-6 rearrangement. Coexpression of CD10 and Bcl-6 is considered to be a marker of a diffuse large B-cell lymphoma arising from follicle centres. Approximately 10% of DLBCL, particularly those with anaplastic morphology, express CD30 and EMA. Bcl-2 protein is expressed in two thirds of cases. The proliferating fraction, as detected by Ki-67 staining, is usually high (more than 60% of neoplastic cells). A proportion of DLBCL associated with Epstein-Barr virus (LMP1+, EBER+) and are mostly observed in patients with immunodeficiencies or in DLBCL resulting from transformation of low grade lymphoma.

Prognosis and predictive factors: A number of features are of value in predicting overall survival and relapse-free survival. Among morphologic variants, a subgroup of T-cell/histiocyte rich DLBCL carries a poor prognosis probably because of an association with adverse clinicopathologic features. DLBCL with expression of full-length AKT also has a poor prognosis. The Ki-67 index is strongly predictive of outcome. Numerous studies have reported a significant effect on disease free survival but not overall survival for DLBCL expressing Bcl-2 protein. The expression of survivin is an unfavorable prognostic factor. In contrast bcl-6 rearrangement is said to be of good prognostic value. The prognostic significance of the Ki-67 index is controversial. The most interesting new advance in DLBCL has come from gene expression studies using DNA microarray technology. In their study, Alizadeh et al identified two distinct classes of DLBCL as germinal centre derived and activated with different clinical courses. In a recent study, using oligonucleotide microarray gene expression, Shipp et al showed that three genes (NOR1, PDE4B and PKC-b) that regulate apoptotic responses to antigen-receptor signaling predicted the outcome of DLBCL. It is likely that this approach will lead to an improved understanding of this biologically heterogeneous group of lymphomas.
RITUXIMAB IN THE TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMAS

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Monoclonal antibodies were first utilized for treatment of lymphoma patients approximately 7 years ago, and were first developed for low-grade or indolent lymphomas. Rituximab was the first monoclonal antibody developed shown to have independent activity against B-cell lymphomas. In vitro data have shown that the unconjugated antibody may induce lymphoma cell lysis through activation of the complement cascade (CDC), activation of immune cells through the Fc fixation (ADCC), induction of apoptosis, inhibition of cell proliferation, and synergism with standard chemotherapy drugs. The first study evaluating response rate to rituximab in patients with aggressive lymphoma (DLCL or mantle cell lymphoma (MCL)) was conducted in Europe three years earlier. For the German High-Grade Non-Hodgkin-Lymphoma Study Group (DSHNL) achieved a significant improvement of treatment results by the unique report on pseudoperipheral T cell lymphoma for both disorders but do not provide any argument in favour of the hypothesis that HRTR-BCL develops out of paragranuloma.

The demonstration that the 25-year old CHOP regimen (McKelvey et al. 1993), which resulted in improvement of treatment results in the other patient subpopulations have not been tested in elderly patients, it is only justifiable to treat these patients outside the setting of a prospective trial. While CHOP-21 is formally still the standard regimen in this group, CHOP-14 could be justified for the rare young patient who does not qualify for such a trial due to the excellent tolerability of CHOP-14 in young low-risk patients and the superiority of the 2-weekly CHOP in the elderly.

T CELL RICH B CELL LYMPHOMA

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The term "T cell rich B cell lymphoma", introduced in 1988, was meant to apply to those B cell lymphomas that show some or all of the features commonly associated with T cell neoplasms. Because immunohistochemistry was a very new technique at that time, it was important to avoid an erroneous diagnosis of T cell lymphoma for these cases. During the following period several studies pointed towards the heterogeneity of T cell rich B cell lymphoma, in particular from a clinical point of view. As more cases of large B cell lymphoma featuring numerous reactive T cells were collected, it became clear that two or more disease entities might be included within this heterogeneous group of lymphomas. On the one hand several cases—emplified by the original report on pseudoperipheral T cell lymphoma by Jaffe et al in 1984—are associated with a typical follicular lymphoma suggesting a germinal centre origin. The demonstration of bcl2 rearrangements in some of these tumours corroborates with this notion. On the other hand, a subgroup of cases characterised by the presence of a prominent histiocytic proliferation in addition to a substantial number of T cells, may be identified. In 1992 we reported on six such cases which suggested that "histiocyte rich T cell rich B cell lymphoma" (HRTR-BCL) may be identified as a distinct clinicopathological entity within the group of T cell rich B cell lymphomas.

Recently we analysed a series of 60 HRTR-BCL in order to provide a detailed morphological and immunophenotypical profile of this disease. This study demonstrates that the lymphoma is characterised by (1) a diffuse or vaguely nodular growth pattern, (2) presence of a small number of CD15−, CD20+ large neoplastic cells, (3) a prominent stromal component composed of both mononuclear and histioocyte, and (4) the scarcity of small reactive B cells. Based on the detailed clinical data available in 40 of these cases the recognition of HRTR-BCL as a clinicopathological entity may be justified as the disease typically affects middle-aged male patients presenting with advanced-staged disease that is not adequately managed with current therapeutic strategies. HRTR-BCL may especially be difficult to differentiate from Hodgkin’s lymphoma, in particular the nodular lymphocyte predominant, paragranuloma type and the more recently recognised lymphocyte rich classical variant.

From a pure morphological point of view distinguishing between diffuse paragranuloma and HRTR-BCL appears virtually impossible. In fact some cases initially diagnosed as diffuse paragranuloma present in an unusually advanced clinical stage and respond poorly to treatment regimens for Hodgkin’s disease, suggesting a diagnosis of HRTR-BCL. Then again few patients may present a morphological picture of HRTR-BCL but have only limited stage disease and respond surprisingly well to treatment. Hence for these few cases where the distinction by morphology alone remains problematic, it may rely above all upon a sound communication between pathologists and clinicians. Finally one might speculate about the relationship between HRTR-BCL and paragranuloma. We studied a series of paragranuloma cases and HRTR-BCL using comparative genetic hybridisation as a method to analyse DNA extracted from single microdissected tumour cells. Data from this study reveal a very complex pattern of abnormalities in paragranuloma with a high number of imbalances per case, in contrast to a simpler one in HRTR-BCL. Nevertheless both conditions show abnormalities at chromosome 1 and 19, rarely found in non Hodgkin’s lymphomas. These results support a common origin for both disorders but do not provide any argument in favour of the hypothesis that HRTR-BCL develops out of paragranuloma.

Key references
**Background:**
In order to evaluate the clinical and prognostic relevance of T-cell-rich B-cell lymphoma (TCRBCL), we performed a matched-control analysis.

**Methods:**
50 cases out of 3500 patients included in the LNH93 and LNH98 studies for aggressive lymphoma were considered as "true" TCRBCL by a panel of 4 pathologists. A control group of 122 patients with "classical" diffuse large cell lymphoma (DLCL) was selected by matching with patients treated in the same protocols.

**Results:**
Histological characteristics of the 50 TCRBCL cases showed diffuse architecture in 29 cases, and areas of nodularularity (not more than 10% of the total tumor area) in 11 cases, whereas 10 cases could not be classified due to the size of the biopsy. Phenotypic analysis showed CD20 positivity on large atypical cells in all cases, positivity of EMA in 34/38 cases (86%), CD30 in 2/38 cases and CD15 in 4/38 cases. In 10/38 cases (26%), small reactive lymphocytes were CD3 positive in all cases, whereas in the nodular areas, small lymphocytes were predominantly CD20+. Bone marrow involvement and/or splenomegaly were present in 31% and 60% of TCRBCL cases, respectively. TCRBCL patients were more frequently staged III-IV (80%). There was no statistically significant difference in terms of survival between TCRBCL and classical DLCL, despite a trend toward a better response (achievement of complete remission) for patients with classical DLCL (p = 0.03).

**Conclusions:**
Our data show that (i) TCRBCL has a particular histopathological and clinical presentation (ii) there is no reason to separate TCRBCL from classical DLCL in terms of therapeutic strategy and management of patients.
was seen in 92% of BL and 50% of DLBCL. True plasmacytoid differentiation was seen only in a few immunoblastic lymphomas.

**Conclusion:** Most pediatric B-NHLs are bcl-2 negative and bcl-6 and CD10 positive. They rarely show plasmacytoid differentiation. They mimic the physiological immunophenotype of germinal center B cells, rendering a direct germinal center derivation of these tumors probable. The immunophenotype of pediatric B cells differs from that of adult DLBCL in that they less frequently express the known adverse prognostic factor bcl-2, whereas they more frequently express CD10 and bcl-6.

043 RELATIONSHIP BETWEEN CYTOGENETICS AND CD10 EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA

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**Introduction:** The frequency of t(14;18) in de novo diffuse large B-cell lymphoma (DLBCL) and its relationship to CD10 expression remains unclear. Moreover, the accuracy of CD10 immunostaining vs flow cytometry has not been systematically studied.

**Methods:** We studied 170 cases of de novo DLBCL for the t(14;18) using standard cytogenetics and 2-color FISH (Vysis). CD10 immunostaining was routinely performed using antibody clone 56G6 (Novo- castra).

**Results:** Of the 170 cases, 44 (26%) had evidence of a bcl-2 rearrangement. CD10 immunostaining was performed on 115 cases, of which 59 (51%) were CD10+ and 36 (49%) CD10−. Among the 59 CD10+ cases, 27/31 cases with a bcl-2 rearrangement were CD10+ (87%), while only 4/31 were CD10−. 32 of the 59 CD10+ cases had no evidence of a bcl-2 rearrangement (54%). Both cytogenetics and FISH were performed in the 115 case subset. FISH identified a bcl-2 translocation in 3 cases negative by cytogenetics and only failed to detect a single cytogenetically positive case 2 cases that were negative by immunostaining were CD10+ by flow cytometry. However, flow cytometry failed to detect 13/59 CD10+ cases, including 8 in which no b-cell clone was detected using light chain restriction criteria.

**Conclusions:** We found t(14;18) in 26% of de novo DLBCL with a clonal karyotype. Although most cases with a bcl-2 rearrangement are CD10+, 54% of the CD10+ cases had no evidence of a t(14;18). Paraffin immunostaining detects most cases with CD10 expression, while flow cytometry may be negative due to the inability to detect a B-cell clone.

044 HIGH NUMBERS OF GRANZYME B POSITIVE TILs IN NODAL DIFFUSE LARGE B-CELL LYMPHOMAS PREDICT POOR CLINICAL OUTCOME

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**Results:** In 1995; Kroft et al demonstrated in Hodgkin’s disease and in anaplastic large cell lymphoma (ALCL), a preferential use of IgVH4–34 (4/7). Clinical data exhibited no significant difference between CD5+ and CD5− DLBCL. FISH analysis revealed no t(14;18)-deletions; the prevalence of deletions of D13S25, and results were compared to those from 55 CD5− DLBCL. On histology, 11/13 cases were frank DLBCL, whereas two were cytologically reminiscent of a blastoid MCL variant. All cases were CD20+, CD5−, bcl-2 and bcl-6 positive (in at least 10% of cells), and negative for CD23 and cyclin D1. One case coexpressed CD10, 11/13 (85%) tumors exhibited complex karyotypic alterations. All cases were t(11;14)- and t(14;18)-negative, whereas the t(3;14) was found in only one case and del(11)(a22a24) was the sole recurrent structural aberration in two cases. CD5+ and CD5− DLBCL revealed no significantly different spectrum of genetic imbalances.

Conclusions: The strong association of D13S25-deletions with B-CLL, MCL and CD5+ DLBCL may indicate that this aberration hits a common precursor cell, the B1 lymphocyte, at a similar stage. The presence of additional P16INK4a-deletions, commonly associated with lymphoma progression, could suggest that additional transforming events took place before the (clonal) expansion of a low grade lymphoma.

045 CYTOGENETIC ANALYSIS OF DE NOVO CDS POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA (CDS+DLBCL)

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CD5 positive diffuse large B lymphomas (DLBCL) may represent a significant entity of non-Hodgkin’s lymphomas with respect to immunophenotype and genetics. Thirteen de novo CD5+ and t(11;14)−negative DLBCL without history of preceding low grade disease were characterized for their clinical, histomorphological, immunohistochemical and genetic findings including IgVH-status, classical and interphase FISH; immunophenotype (BCL1, BCL2, BCL6, CD3, CYCD1, CD23, ATM, TP53) and results were compared to those from 55 CD5− DLBCL.

**Results:** We found t(14;18) in 26% of de novo DLBCL with a clonal karyotype. Although most cases with a bcl-2 rearrangement are CD10+, 54% of the CD10+ cases had no evidence of a t(14;18). Paraffin immunostaining detects most cases with CD10 expression, while flow cytometry may be negative due to the inability to detect a B-cell clone.

**Conclusion:** The presence of many granzyte B+ TILs in pre-treatment biopsies of DLBCL is a strong marker for a poor clinical outcome, independent from stage and IPI. In the future we will investigate whether this observation results from acquired cross-resistance of tumour cells to both CD10 induced apoptosis and chemotherapy induced cell death.

046 DE NOVO CDS+ AGGRESSIVE B-CELL LYMPHOMA: A PATHOLOGICAL STUDY OF 29 CASES WITH IDENTIFICATION OF A NEW FOLLICULAR LYMPHOMA VARIANT

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**Background:** CD5+ aggressive B-cell lymphomas are a quite rare and poorly characterized group of non-Hodgkin lymphomas. So far, few studies have analyzed the clinicopathological features of this heterogeneous group of lymphomas (Matolcsy et al, Am J Pathol 1995; Kroft et al, Am J Clin Pathol 2000). Interestingly, CD5+ cases expressed on DCL were usually correlated with a worse clinical outcome.

**Purpose:** To assess the histological, phenotypic and molecular features of CD5+ aggressive B-cell lymphomas. 29 cases were collected from our institution in the period 1987–2000 excluding those with previous diagnosis of low grade lymphoma or Richter’s syndrome. Immunohistochemistry included anti-CD3, CD20, CD5, CD10, cyclin D1, Bcl2, CD21, CD23 and MIB-1 antibodies. Flow cytometry analysis was performed on 25 out of 29 cases. PCR-based molecular analysis was carried out on either frozen or formalin-fixed tissues on 23 cases.

**Results:** All the cases were CD20+, CD3−, CD5+ and showed a proliferation rate higher than 35% with the MIB-1 antibody. 12/29 cases expressed cyclin D1 and 20/29 cases expressed cyclin D2. 23/29 cases were cyclin D1+ and another 4
cases were classifiable as CD5+, CD10+ grade IIIb follicular lymphomas by morphology, follicular pattern with variable degree of diffuse component, immunohistochemistry (Bcl-2+ and CD21+ CD23+ evident follicles) or molecular biology (Bcl-2 rearrangement). The remaining 11 cases lacked a specific phenotype or markers and were classified as CD5+ B DCL.

Conclusions: The characterization of de novo CD5+ aggressive B cell lymphomas showed that the majority were either MCLs or DCLs. In addition and more interestingly, we provide evidence for a CD5+ variant of grade IIb follicular lymphoma. Clinical correlations are needed to assess the importance of this phenotypic variant.

047 VERY LATE RELAPSE IN DIFFUSE LARGE B-CELL LYMPHOMA RARELY REPRESENTS A SECOND MALIGNANCY AND IS OFTEN ASSOCIATED TO TRANSFORMED FOLLICULAR LYMPHOMA

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Background: About 50% of patients with diffuse large B-cell lymphoma (DLBCL) show relapse and ultimately die of their disease. Although the vast majority of relapses occur within the first 3 years after presentation, later and very late relapses may occur. Little is known about the biological background of these late relapsing DLBCL.

Material/Methods: From the clinical files of the Netherlands Cancer Institute, 20 cases of patients with DLBCL who presented with a relapse after 4 years disease-free interval were selected. All cases were reviewed and reclassified according to the WHO-classification. Standard immunohistochemistry was performed, including CD10, bcl-2 and bcl-6. Clinical data were collected. DNA was isolated from representative biopsy samples of the initial episode and the relapse. Immunoglobulin-heavy (IgH) chain gene CDR2 and CDR3 regions were amplified and monoclonal rearranged products were sequenced. Bcl-2/IgH translocation products were studied using a PCR-assay for translocation in the bcl-2-MBR and bcl-2-MCR regions.

Results: The mean interval between first presentation and relapse was 87.3 months (range 40–207). In 7/12 patients, IgH-genes could be successfully amplified and sequenced for both episodes. In 6 cases, identical VDJ-complexes were seen with evidence of ongoing VH-mutation over time and in one case, an identical MCR-bcl-2/IgH translocation was demonstrated, both indicative of a clonal relation (intervals 52–144 mo). Different VDJ-complexes were found in one case (intervals 207 mo), suggesting an independent second DLBCL. The MBR-bcl-2/IgH translocation complex present at presentation was not reproducible at relapse, suggestive, but not conclusive for an unrelated process (interval 40 mo). The clonal relation will be further studied using loss-of-heterozygosity patterns. In 5 of the clonally related cases and one additional unresolved case, consistent expression of CD10 (r14;18) or a persistent monoclonal small B-cell component after treatment of the relapse was suggestive of a relation with transformed follicular lymphoma.

Conclusions: Relapsed disease in DLBCL after more than 4 years up to 12 years disease-free interval is still related to the original clonal disease and can often be explained by two transformed episodes of an underlying, but cryptic follicular lymphoma. Development of a second, clonally independent DLBCL is a very rare event and may be therapy-induced.

048 PROGNOSTIC SIGNIFICANCE AND MORPHOLOGICAL FEATURES OF DIFFUSE LARGE B-CELL LYMPHOMA EXPRESSING CD10

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Diffuse large B-cell lymphoma (DLBCL) is a heterogenous group of lymphomas and determination of biological prognostic factors that could predict outcome is of major interest. CD10 antigen expression in DLBCL has already been reported ranging from 20% to 56% of cases. There is a controversy concerning its prognostic impact. CD10 expression is considered as a good marker of centrofolicular-derived DLBCL. The aim of this study was to determine among 98 patients, enrolled in the LNH 93 protocol and all treated with the reference ACHVPH arm, the expression of CD10 by immunohistochemistry. This was correlated with clinical and pathological characteristics. Morphological analyses have been evaluated independently by two pathologists. The presence or less than 50%) or absence of immunoblasts as well as multilobated cells (more or less than 30%) were evaluated. Each case was classified according to updated Kiel classification (immunoblastic, centroblastic monomorphic, centroblastic polymorphic, multilobated). The main initial characteristics of these patients were: median age: 56 y; LDH≤1N: 55%; III–IV stage: 56%; ECOG=1: 21%; extranodal sites >1: 23%. Of the 98 patients studied, 33 (34%) expressed CD10. Overall survival rate and event-free survival were not significantly different according to CD10 expression (p = 0.44, respectively). There was no correlation between clinical parameters, IPI risk groups, and CD10 expression with the exception of LDH levels. Indeed, among the CD10 positive cases, only 36% had LDH>1N (p = 0.005). Among the 91 cases evaluable for the morphological analysis, 18% did not contain any immunoblasts and 23% disclosed more than 50% immunoblasts. Numerous multilobated cells (more than 30%) were present in 26% of the cases. There was no correlation between CD10 expression and the presence of numerous multilobular cells (p = 0.14) or diagnosis of immunoblastic lymphoma (p = 0.43). There was a tendency of rare CD10 expression among the cases with a majority of immunoblasts (5/21) compared to cases without any immunoblasts (8/16), but not significant (p = 0.09).

Therefore, it appears that CD10 expression does not influence survival or event-free survival in DLBCL. Moreover, we show that centrofolicular-derived DLBCL may present with numerous immunoblasts or as an immunoblastic lymphoma.

049 QUANTITATIVE IMMUNOHISTOCHEMICAL ANALYSIS OF BCL-6 PROTEIN EXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: BCL-6 is a 95-kDa protein, normally expressed in germinal B-cells, that has been demonstrated to function as a sequence specific transcriptional repressor. A majority of diffuse large B-cell lymphomas have been shown to harbor BCL-6 gene mutations (70%) and/or rearrangements (40%). Chromosomal translocations have been reported to deregulate BCL-6 gene expression. We have previously demonstrated that patients with high BCL-6 gene expression as evaluated by real-time reverse transcription-polymerase chain reaction (RT-PCR) assay treated with anthracycline-based chemotherapy regimens have longer median overall survival times. We demonstrated a similar correlation between BCL-6 protein expression and overall survival by visual estimation of anti-BCL-6 immunostains performed on a subset of our original cases.

Purpose: The purpose of this study is to determine whether quantitative immunohistochemical analysis of BCL-6 protein expression using the Chromavision Advanced Cellular Interpretation System (ACIS) correlates with BCL-6 mRNA expression and to determine whether this method is useful as a surrogate predictor of median overall survival in an independent group of 42 patients with diffuse large B-cell lymphoma.

Results: The level of BCL-6 protein expression as evaluated by ACIS quantitative immunohistochemical analysis correlates well with BCL-6 mRNA expression as determined by real-time RT-PCR (r = 0.592, n = 18) and is comparable to visual estimation (ACIS r = 0.736, visual r = 0.805). In addition, patients with tumors showing >15% total nuclear staining for anti-BCL-6 as evaluated by ACIS analysis have a longer median overall survival time than those with >15% total nuclear staining [unreached versus 58 months]. There is no correlation between BCL-6 expression and tumor growth fraction as determined by quantitative immunohistochemical analysis for Ki-67 (r = 0.051).

Conclusion: We conclude that quantitative immunohistochemical analysis is a reliable method for determination of BCL-6 expression in diffuse large B-cell lymphoma.

BCL-2 AND BCL-6 EXPRESSION PROFILE STRATIFIES PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA (DCLL) PATIENTS INTO DISTINCT PROGNOSTIC GROUPS

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Aims: To assess the prognostic significance of approximate immuno-histochemical surrogate markers for follicle centre (FC) (Bcl-6 and

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CD10 and activated B-cell (ABC) phenotype (CD138 and Bcl-2) in DLCL since mRNA expression studies have shown a better prognosis for FC compared to ABC DLCL.

**Methods:** Paraffin sections from 42 DLCL of patients enrolled between age 20 and 67 from 1991 to 1994 in a prospective trial of high dose therapy for primary aggressive lymphoma (mean follow-up 70 months), were stained on a Ventana Nexes machine for Bcl-2 (Dako clone 123), Bcl-6 (Dako clone PG-B6), CD10 (Novocastra clone 56C6) and CD138 (Dako clone M15).

**Results:** 43% (18/42) were Bcl-2 positive (+). Strong positivity (+++) was associated with a worse prognosis, 79% (33/42) were Bcl-6++. ++ was associated with a better prognosis. Combining results stratified patients into three groups (p<0.015) with excellent estimated 5-year disease-specific survival (OAS) for Bcl-6+/Bcl-2 weak or negative (+/-) DLCL (8/42), bad OAS for Bcl-6-/-Bcl-2++ DLCL (6/42), and intermediate OAS for double +/ or double ++ DLCL (29/44). 33% (15/44) were CD10+. ++ was associated with a worse prognosis. One lymphoma was CD138+.

**Conclusion:** The Bcl-2/Bcl-6 expression profile stratifies patients with DECL into prognostic groups. FC and paraffin blocks normal apoptosis may convey the excellent prognosis to Bcl-6++. One lymphoma was CD138+.

The morphological spectrum of NBS lymphomas in our patients differed from usual lymphomas spectrum for this age: 8 were DLBCL, 1 Burkitt-like, 1 HD, 3 TBLB/ALL and 1 AILT. This type of lymphoma spectrum is more characteristic of adult than pediatric patients. Among DLBCL there were 3 TCRCL variants and all but 2 contained variable, usually large amounts of T lymphocytes. In all DLBCL cases large cells were CD20, CD79a positive. The pattern of CD10, bcl-2, bcl-6, LMP expression and the amount of positive cells varied considerably making any stratification very difficult. Rearrangeent studies revealed clonal IgH gene rearrangements in all but one DLBCL cases, clonal IgK in all cases and additionally IGL in two. In one DLBCL—apart from K and L rearrangements—there were clonal TCRD and TCRB cells but without TCRG rearrangement. No one case disclosed Bcl-2/17 gene translocation. Rearrangeent studies in TLBL revealed clonal TCRD and TCRB in 2 cases, TCRB and TCRD in 1. The case diagnosed as AILT displayed clonal IgH and IgK rearrangements. Our study underlines the need for the application of wide spectrum IHC, and a wide range of primers in assessing difficulties in the diagnosis of NBS lymphomas. Sequencing of clonal rearrangements and PCR EBV studies are under investigation.

**EXPRESSION OF BMI IN NODAL DLBCL**

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**Background:** Diffuse large B cell lymphoma (DLBCL) is an umbrella term comprises a number of different biological entities that cannot be distinguished by morphological or immunophenotypical analysis. Among the recently described molecules, bmi, a product of the polycomb genes plays a role in germinatal center (GC) development.

**Objectives:** This work aims to investigate the pattern of expression of BMI in DLBCL, to correlate its expression with the histologic subtypes, and with the expression of CD10 and bcl-6.

**Methods:** Paraffin sections from 9 reactive nodes and 42 nodal DLBCL (9 on top of follicular lymphoma (FL), 2 on top of marginal zone lymphoma (nodal MZL), 3 on top of lymphoplasmacytic lymphoma, 28 de novo DLBCL) were immunostained for the expression of bmi. Moderate nuclear staining in more than 10% was considered positive.

**Results:** In reactive nodes, bmi was seen in mantle zone cells and centrocytes, while in DLBCL it was also expressed in centroblasts, plasmacytic cells and immunoblasts. Bmi expression was restricted to 26.19% of DLBCL (11/42); 2/2 of DLBCL on top of nodal MZL, 1/3 of lymphoplasmacytic and 8/28 of de novo subtype. None of the DLBCL on top of FL were stained. The expression was inversely correlated to CD10 (p<0.005) and insignificantly associated with bcl-6.

**Conclusion:** Most bmi+ DLBCL enter in the gray zone, rendering difficult their histogenesis either GC or post GC derived. Bmi+ DLBCL might represent an interesting subgroup for which the study of oncogenes and tumor suppressor genes would be of interest. The prognostic significance of such expression in the 11 cases is currently under investigation.

**Extranodal aggressive B-cell lymphomas**

**AGGRESSIVE B-CELL LYMPHOMAS OF THE MEDIASTINUM**

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The most common lymphomas of B-cell origin arising in the mediastinum are the primary mediastinal B-cell lymphoma (MBL) and Hodgkin’s disease. MBL is a highly aggressive B-cell lymphoma with several specific clinical, morphological, immunological and genetic features. The incidence of MBL is approximately that of Burkitt’s lymphoma or lymphoblastic lymphoma. There is a slight predominance of the female sex. Patients with MBL present with symptoms due to invasion or compression of structures in the upper anterior mediastinum, invasion of lungs or the pericardium frequently occurs, a fact which underlines the aggressiveness of this lymphoma. In recurrent disease, haematogenic spread to parenchymatous organs is also well known. Cytomorphology reveals a broad spectrum, ranging from a medium to large cell variant with a variable background of sclerosis. Histological subtypes have no prognostic significance. MBL is a B-lymphoma with defects in the expression of MHC class I and class II molecules as well as Ig protein, whereas the Ig-associated molecule CD79a is constitutively expressed. Asteroid B-cells within the thymic medulla are regarded as the normal counterpart to MBL. Recent data are suggestive of a post-germinatal centre origin, since the clonally rearranged immunoglobulin genes are hypermutated but do not show evidence of ongoing mutational activity. The cytogenetics of MBL reveal a highly characteristic pattern of genomic aberrations with gains on the short arm of chromosome 9 as the hallmark. Furthermore, some cases show DNA high-level amplifications on 2p involving the REL proto-oncogene, a finding that is frequently encountered in classical Hodgkin’s disease or extranodal diffuse large B-cell lymphoma. Currently, standard treatment strategies do not differ from that applied for DLCL. However, there are preliminary data suggesting a particularly high efficacy of time-intensive therapy regimens in MBL.

Due to these rather unique biological, clinical and molecular cytogenetic parameters, MBL is currently a defined subtype of diffuse large B-cell lymphoma with its own ICD-0 code [i.e. 9679/3]. Interestingly, morphology, localisation, immunology and genetics reveal some features in common with classical Hodgkin’s disease.
During an international co-operative study, 137 primary mediastinal large B-cell lymphomas (PMLBCL) were collected for extensive pathological evaluation. At light microscopy, the neoplasms mostly consisted of large cells with a predominantly diffuse growth pattern and varying degrees of nuclear polymorphism. In 96 instances, they displayed a wide rim of clear cytoplasm. In 35 cases, the cytoplasm was slightly basophilic, while in the remaining 6 it varied from clear to basophilic. The neoplastic growth evoked a fibrinous reaction with frequent compartmentalization. Necrosis, nodularity and Reed-Sternberg-like elements were sometimes detected, as was infiltration of the vessels and/or lung parenchyma. On immunohistochemistry, extensively performed in 80 cases, lymphomatous cells showed the following phenotype: CD45+, CD20+, CD79a+, BSA+, CD30−, HLA-DR+, BOB.1+, Oct-2−, Bcl-2+, MAL protein−/+, Bcl-6+, IFR4+, CD10−, CD21+, and CD3+. The search for immunoglobulins (lg) as well as for the corresponding RNA provided negative results. At molecular analysis, carried out in 45 cases, more than half the tumors displayed BCL-6 gene mutations, which usually occurred along with functioning somatic IgVH gene mutations and Bcl-6 and IRF4 expression. Frequent methylation of the MGMT gene was also observed. The present study significantly expands the spectrum of our knowledge on the tumor. First, it strengthens the notion that PMLBCL is a distinct entity which differs from other diffuse large B-cell lymphomas. Secondly, it suggests that it is often derived from germinal center or post-germinal center cells. Thirdly, it prompts us to investigate further the mechanism by which defective Ig production occurs in spite of an intact transcription machinery and lack of IgVH gene crippling mutations.

Conclusions:

Our study clearly shows that a vast majority (70%) of MBLCB arise from activated dendritic thymic B cells. It is conceivable that a percentage of MLBCL arise from this subpopulation of activated thymic B cells. In our study the vast majority (97%) of MLBCL showed strong expression of CD23, whereas only 14% of non-mediastinal DLBCL showed strong expression of CD23. MLBCL strongly express CD23 whilst the same antigen is expressed in a smaller fraction (5%) of non-mediastinal DLBCL. Accordingly, we can confirm that a substantial number of MBLCB arise from activated dendritic thymic B cells and suggest that CD23 should be included in the panel of antibodies currently used to characterise this subtype of DLBCL.
non-medial diffuse large B-cell lymphomas (NM-DBL). The MAL mRNA was initially identified during T-cell development, and later found in myelin-forming cells and certain polarized epithelial cell lines. It encodes a proteolipid believed to be involved in membrane microdomain stabilization, transport machinery and signal transduction. The aims of our study were to extend MAL expression analysis to B and T cell suspensions from peripheral blood, tonsil and spleen, to normal lymphoid tissues, to 185 lymphomas representing most B, T and Hodgkin lymphoma entities and to the PMBL derived B-cell line MedB-1, using flow cytometry and immunohistochemistry. Our results show that in the normal B-cell compartment, MAL expression is restricted to a minor subpopulation of thymic medullary B-cells, and to occasional mature plasma cells located in the interfollicular areas of tonsil and lymph nodes. In the T-cell lineage, MAL is highly expressed in thymocytes in a large percentage of peripheral CD4 T-cells, and in a lower proportion of CD8 peripheral T cells. Among B-cell lymphomas (n = 110), MAL expression in tumour cells was observed in 21/33 PMBLs (70%) and in 3/5 plasmacytoma/myeloma, but not in all other B-cell lymphomas with the exception of 1/33 NM-DBL. The MedB-1 B-cell line was also MAL positive. Among T-cell neoplasms, MAL was highly expressed in lymphoblastic leukaemia (5/6) whereas mature T-cell lymphomas were essentially MAL negative (27/28). Among 41 Hodgkin lymphomas, 3 nodular sclerosing cases with a large Reed-Sternberg cells and 2 cases with a large mononuclear cell lineage were MAL positive. The MedB-1 B-cell line was also MAL positive. Among T-cell neoplasms, MAL was highly expressed in lymphoblastic leukaemia (5/6) whereas mature T-cell lymphomas were essentially MAL negative (27/28). Among 41 Hodgkin lymphomas, 3 nodular sclerosing cases with a large Reed-Sternberg cells and 2 cases with a large mononuclear cell lineage were MAL positive. The MedB-1 B-cell line was also MAL positive.

**059** TCL1 DOWNREGULATION AND CD27 EXPRESSION IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA WITH SCHLORIS ARE CONSISTENT WITH THEIR POST-GERMINAL-CENTER ORIGIN


**Background:** Mediastinal large B-cell lymphoma with sclerosis (MBL-CLS) is a subtype of diffuse large B-cell lymphoma (DLBCL) with distinctive clinicopathological and biological features. Recent immunohistochemical and molecular-genetic evidence has strongly suggested an origin of MCL-CLS from B-cells at the germinal-center (GC) or post-GC stage of differentiation. TCL1 is an oncogene involved in the pathogenesis of T-cell prolymphocytic leukemia. Furthermore, in normal conditions TCL1 is expressed in naive B-cells, mantle cells and some GC-cells and down-regulated in most marginal zone B-cells, plasma cells and in all memory B-cells of peripheral blood (PB). On the contrary, CD27 is expressed in all PB memory B-cells and in most marginal-zone B-cells. Thus, the expression of TCL1 and CD27 seems to be mutually exclusive. Furthermore, studies have demonstrated TCL1 expression in a large series of pre-GC and GC-derived B-cell lymphomas, including diffuse large B-cell lymphoma, and its downregulation in post-GC neoplasias.

**Aim/Methods:** On these grounds, we have investigated the expression of TCL1 (clone 27D6/20) and CD27 (clone M727-1) in PB of normal donors, in reactive hyperplastic lymphoid tissues from human tonsils and in a series of 22 well-characterised cases of MCL-CLS, using immunohistochemistry (an formalin-fixed, paraffin-embedded tissue sections) and flow-cytometry, with the aim to obtain additional information about the pathogenesis of this type of lymphoma.

**Results:** Flow cytometry analysis, in normal PBL as well as in cellular suspensions from hyperplastic tonsils, demonstrated that TCL1 and CD27 were never co-expressed. 22/22 cases (100%) of MCL-CLS were TCL1 negative. 5/16 cases (31%) showed variable expression of CD27.

**Conclusions:** (1) Our findings are consistent with the hypothesis that MCL-CLS are derived from a subset of post-GC B-cells; (2) Furthermore, the comparative investigation of TCL1 and CD27 expression seems to be an original approach for studying the differentiation stage of B-cells in lymphoproliferative disorders.

**060** T(11;18)(Q21;Q21): DETECTION BY RT-PCR FROM FORMALIN FIXED TISSUES AND ITS VALUE IN PREDICTION OF GASTRIC LYMPHOMA TO HELICOBACTER PYLORI ERADICATION


Eradication of Helicobacter pylori leads to complete regression of gastric MALT lymphoma in 75% cases. To determine whether a lymphoma responds to the therapy, prolonged follow-up is essential. Clinical staging is helpful in prediction of the response of lymphomas at stage IE or above, but not those at stage IE, which account for the majority. In a small series of cases, we showed that T(11;18) positive MALT lymphomas failed to respond to H. pylori eradication. However, this finding remains to be validated in a large cohort, particularly in stage IE tumours. We developed a RT-PCR method for detection of T(11;18) from archival formalin-fixed and paraffin-embedded tissues and evaluated its prognostic value in 111 patients with H. pylori-positive gastric MALT lymphoma treated with antibiotics. Clinical staging was carried out in each case prior to therapy. The response of lymphoma to H. pylori eradication was determined by repeated endoscopy and histological examination of gastric biopsies. Tissue specimens from diagnostic biopsies were analysed for T(11;18) by RT-PCR of the API2-MALT1 fusion transcript. 47 of the 48 patients who showed complete regression had lymphoma at stage IE, while 43 of the 63 cases who showed no response also had stage IE tumours and the remaining cases had stage IIE or above. T(11;18) was detected in 2 of the 48 complete-regression cases and these positive cases showed lymphoma relapse in the absence of H. pylori re-infection. In contrast, the translocation was present in 42 of the 63 (67%) no-response cases, including 26 of the 43 at stage IE. T(11;18)-positive gastric MALT lymphomas including those at stage IIE do not respond to H. pylori eradication. Detection of T(11;18) should help clinicians to choose the optimal therapy for patients with gastric MALT lymphoma.

**061** PRIMARY CUTANEOUS FOLLICULAR LYMPHOMA: A CLINICOPATHOLOGICAL AND MOLECULAR STUDY IN SUPPORT OF A DISTINCT ENTITY

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**Background:** Primary cutaneous B-cell lymphomas displaying a follicular growth pattern are rare and remain poorly defined, particularly in terms of the frequency of detection of t(14;18) and whether or not they are a homogeneous group, they represent an entirely distinct entity from follicular lymphoma (FL) arising in lymph nodes.

**Purpose:** To define the morphologic, immunophenotypic, clinical and genetic features of 16 cases of primary cutaneous FL and to compare findings with those of stage 1 FL arising in lymph nodes and FL secondarily involving the skin.

**Results:** All cases of primary cutaneous FL displayed a follicular architecture and a CD10 and/or bcl-6 positive phenotype, with 13/16 cases also expressing bcl-2 protein. None harboured t(14;18) when assessed by PCR using primers to the major breakpoint cluster region, a significantly different finding compared with cases of stage 1 nodal FL (p <0.001) and secondary cutaneous FL (p <0.039).

Despite a propensity for relapse similar to stage 1 nodal FL, the group of primary cutaneous FL were significantly more likely to attain complete remission when last seen compared with 49 out of 87
patients with stage I nodal FL (p <0.005). No lymphoma related deaths were observed in 15 cases with a mean follow up greater than 60 months (range 5–119 months).

Conclusions: These results support the concept of a subtype of FL lacking t(14;18) involving the major breakpoint cluster region, and with a propensity to arise in the skin. Despite a high relapse rate patients with cutaneous FL are more likely to achieve complete remission and may ultimately have a more favorable long-term prognosis than those with equivalent nodal disease.

[062] PRIMARY CUTANEOUS LARGE B-CELL LYMPHOMA OF THE LEG: HISTOGENETIC ANALYSIS OF A CONTROVERSIAL CLINICO-PATHOLOGIC ENTITY

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Background: Primary cutaneous B-cell lymphoma of the leg (PCBCL-leg), recently included in the E.O.R.T.C. classification of primary cutaneous lymphomas diagnosed in the spleen. No patient had a previous diagnosis of a lymphoproliferative disease at diagnosis.

Results: The clinical, morphological, immunohistochemical and molecular features of 5 cases of PCBCL-leg were analysed, with special regard to various phenotypic and genotypic markers, including mutations of the Ig and of BCL-6 genes, as well as expression of the bcl-6, MUM1 and CD138/syndecan-1 proteins.

Results: All the cases resembled in morphology DLBCLs and immunohistochemically, all stained for the L26/CD20oc and CD79a antigens, expressed the bcl-2, bcl-6 and MUM1 proteins but were negative for both the CD10/CALLA and CD10 antigens. With respect to molecular analysis, in all tested cases, the lymphoma population carried hypermutation of Ig genes and all but one case also harbored mutations of the BCL-6 gene; in 3/5 cases we found an aberrant methylation of the MGMT-gene, which codes for an enzyme that protects cells from the toxicity of environmental and therapeutic alkylating agents.

Conclusion: Our results indicate that the morphological, immunohistochemical and molecular profiles of PCBCL-leg are similar to the majority of DLBCLs at other sites. Therefore, caution seems justified before definitely considering PCBCL of the leg as a distinct entity.

[063] SPLENIC LARGE B CELL LYMPHOMA: A CLINICOPATHOLOGIC STUDY

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Background: Although the spleen is frequently found to be involved in extranodal marginal zone lymphoma, the diagnostic criteria of large B cell lymphoma (LBCL) is rarely established initially in a spleenectomy specimen. We review here the clinical, morphological, immunohistochemical and molecular features of a series of 34 large B cell lymphomas diagnosed in the spleen. No patient had a previous diagnosis of lymphoma and none presented with peripheral lymphadenopathy at diagnosis.

Design: Paraffin embedded tissue sections of splenic LBCL were immunostained for CD20, CD3, IgD, CD10, bcl2, bcl6, cyclin D1, p16 and p53. Clinical data were reviewed. t(14;18) was studied by PCR.

Results: Three morphological patterns of splenic involvement were identified. (a) A common macroscopic pattern of splenic involvement was an uni a multinodular mass (21 cases) composed of sheets of large B cells. The immunophenotype was CD10 + in 7/21 cases, bcl2 + in 9/21 cases, bcl6 + in 18/21 cases, IgD + in 6/21 cases, with overexpression of p53 in 7/21 cases. 11 patients were in clinical stage II-h, and 11 in clinical stage III. In 8 cases, t(14;18) was absent by PCR. 13 patients were alive after a follow-up of 7–71 months after diagnosis, whereas 3 patients died at 1, 6 and 24 months post-splenectomy. In 9 cases, a micronodular pattern was observed. Small nodules involving the entire parenchyma were centered in the white pulp, with no variable red pulp involvement. These nodules varied in size, occasionally coalescing. The immunophenotype was CD10 + in all cases, bcl2 + in 7/9 cases, bcl6 + in 8/8 cases, IgD + in 4/9 cases, with p53 overexpression observed in 1/9 cases. In 3 cases, the histological features were consistent with those described for T-cell rich B cell lymphoma. None of 6 cases studied for t(14;18) was positive. 6/8 patients were in clinical stage IV, and 2 cases in clinical stage I. Six patients died of tumor after a follow-up of 3–30 months, and 2 patients were alive with disease at 10 and 12 months respectively. (a) More rarely, predominantly diffuse red pulp involvement was observed (4 cases). The immunophenotype was CD10 + in 2/3, bcl2 + in 2/4, bcl6 + in 1/1, IgD + in 3/4, with high p53 expression in 2/4. The 4 cases were diagnosed in clinical stage IV, 3 of them dying at 11, 30 and 36 months respectively.

Conclusion: Large B cell lymphoma presenting in the spleen is characterized by a marked degree of morphologic and clinical heterogeneity. Cases with nodular pattern have a more favorable clinical course that cases with diffuse and micronodular pattern (p <0.05). Phenotypic or molecular specific features of these varieties need to be identified.

[064] THE PLASMABLASTIC VARIANT OF DIFFUSE LARGE B-CELL LYMPHOMA IS ASSOCIATED WITH POOR CLINICAL OUTCOME

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Purpose: To define reproducible criteria for the plasmablastic morphologic variant of diffuse large B-cell lymphoma (PB-DLBCl).

Patients/Methods: Sixty-six DLBCL were reassessed and categorized as either centroblastic (CB), immunoblastic (IB) or PB-DLBCl applying standardized morphologic criteria. Blinded samples were reviewed by three independent pathologists. The final consensus classification included 44 CB (67%), 7 IB (10%), and 15 PB-DLBCl (23%).

Results: The reproducibility between two centers (Vienna, Würzburg) was 93.5%. PB-DLBCl were CD20+, clgM+, MUM-1+, CD138+, bcl-6+, corresponding to an activated B-cell phenotype. Immunoglobulin VH gene mutation analysis was consistent with a germin or post-germineral cell origin. By FISH-analysis, 11/13 (85%) PB-DLBCl had a monoallelic TP53-deletion, whereas results from the TP53 specific probe in CB (n = 7) or IB (n = 3) were normal. Pretreatment characteristics of patients with PB-DLBCl included a tendency for more B-symptoms, extranodal disease and a higher IPI. However, none of these clinical features was significantly different from the other DLBCLs. The poor response to treatment of patients with PB-DLBCl indicated biological resistance to standard chemotherapy with a low CR-rate (47%) and a high relapse rate (71%). The disease was also resistant to autologous stem cell transplantation. Most importantly, the median overall survival was 14 months; CR <0.002 and disease-free survival (3 months; p <0.002) was significantly shorter compared to CB and IB DLBCL patients.

Conclusion: Our data indicate a strong association of plasmablastic morphology with poor response to chemotherapy and short survival. We therefore propose to evaluate our criteria and test them in large clinical trials.

[065] MUTUAL EXCLUSION OF t(11;18)(q21;q21) AND NUMERICAL CHROMOSOMAL ABERRATIONS IN GASTRIC LYMPHOMAS

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Gastric non-Hodgkin lymphomas (NHL) can be divided histologically into low-grade (IG) and high-grade (HG) extranodal marginal zone

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lymphoma of MALT-type and diffuse large B-cell lymphoma (DLBCL). The translocation t(11;18)(q21;q21) is the most frequent structural chromosomal aberration in MALT-type lymphomas, but the precise frequency and its relation to chromosomal instability among the different types of gastric B-cell lymphomas has yet to be determined. We studied the incidence of t(11;18)(q21;q21) and numerical aberrations of selected chromosomes in 36 LG and 39 HG gastric MALT-type lymphomas and 12 gastric DLBCL by dual-color interphase fluorescence in situ hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR). t(11;18) was exclusively detected in LG MALT tumors (FISH 22%; RT-PCR 24%) and was absent in HG MALT lymphoma and DLBCL. Sequence analysis of the heteroduplex-forming cDNAs revealed breakpoints both in the API2 gene and the MALT1 gene. t(11;18)(q21;q21) appeared to be the sole genetic abnormality in all of the t(11;18)-positive lymphomas studied. In contrast, 45% of the t(11;18)(q21;q21) negative LG MALT lymphomas showed trisomies specifically of chromosome 3 and 18. In HG MALT lymphomas with separate low- and high-grade tumor components some trisomies were detected in both components, whereas others occurred only in the high-grade tumor cells. Our results indicate that LG MALT lymphoma can be divided in lymphomas characterised by the t(11;18)(q21;q21) which are unlikely to transform into high-grade tumors and t(11;18)(q21;q21)-negative LG MALT-type lymphomas that may develop into HG MALT after acquisition of additional genetic aberrations.

Introduction: Extraluminal marginal zone lymphomas of MALT type show an indolent clinical behaviour, and often prolonged localization to the site of origin, but some may eventually disseminate to other mucosa-associated sites or adjacent lymph nodes. The translocation t(11;18)(q21;q21) which results in the API2-MALT1 fusion, is found in 20–50% of the examined tumors and is the most frequent structural abnormality associated with these lymphomas. Here we study the clinical significance of molecular monitoring of tumor cells in peripheral blood (PB) by clone-specific PCR in t(11;18)+ and t(11;18)− MALT lymphoma patients, during the course of disease.

Patients/Methods: Endoscopic biopsies and peripheral blood samples were selected 3 months after initial diagnosis of four patients with gastric lymphoma (3 low-grade [LG] tumors; 1 high-grade [HG] tumor), one patient with conjunctival LG MALT lymphoma and one patient with gastric Burkitt lymphoma. Follow up samples were taken between 0 to 30 months after initial diagnosis in 3 cases. All lymphomas showed monoclonality in CDR3-PCR analysis. The presence of t(11;18)(q21;q21) in the MALT lymphomas was determined by API2-MALT1 RT-PCR. The immunoglobulin heavy chain sequences of malignant clones in patient biopsy samples were determined and allele-specific oligonucleotides (ASO) were designed for sensitive real-time ASO-PCR detection (ABI Prism 7700 sequence detector) of tumor cells in PB.

Results: Tumor clones could be monitored in PB of a patient with Burkitt lymphoma and in PB of two patients with gastric LG MALT lymphoma but were not detected in three MALT lymphoma patients (1 LG conjunctival, 1 LG and 1 HG tumor). Interestingly, both LG MALT lymphoma patients with malignant clones in PB were found to be t(11;18)(q21;q21) and did not respond to Helicobacter pylori eradication therapy. Two patients with clone-negative PB lacked the translocation, while in PB of one t(11;18)(q21;q21) containing LG MALT lymphoma, no tumor clone could be detected. One t(11;18)(q21;q21) lymphoma patient showed complete agreement between the presence of tumor cells in PB and the histology in biopsies during follow up at 7, 11, 17, 24 and 30 months after initial diagnosis.

Conclusion: These findings indicate that t(11;18) MALT lymphomas are not always clonal, may be more advanced tumors which do not respond to local H. pylori eradication therapy. Monitoring tumor cells in peripheral blood follow up samples (either with CS-PCR or API2-MALT1 RT-PCR) of patients with these tumors may verify the efficacy of treatment regimens during the course of disease and might be an alternative to diagnosis of endoscopic biopsies.

**067 IMPROVED B-CELL CLONALITY ANALYSIS IN GASTRIC MALT LYMPHOMAS USING RTTH DNA POLYMERASE**


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Background: Polymerase chain reaction (PCR)-based detection of immunoglobulin heavy chain (IGH) gene rearrangement for determination of B-cell clonality needs to be simple, but optimally sensitive and reproducible. Efficient IGH PCR analysis can be hampered by sequence variability in the template DNA, despite the use of degenerative primers.

Purpose/Methods: To improve the reliability of the method, we have tested an enzyme blend (rTth DNA Polymerase, XL) providing increased primer matches during PCR and thereby increases the chance of amplification of a clonal IGH rearrangement. After a simple and fast DNA isolation, the clonality was determined by FR3 single and FR2 seminested protocols. In addition, 4 template DNA dilutions were used to discriminate between false positive clonal patterns and true monoclonality. The activity of the rTth and conventional Taq enzymes was compared in routinely processed endoscopic biopsies of 15 primary gastric MALT lymphoma cases.

Results: Using rTth, FR3 and FR2 PCR products were obtained in 60% (9/15) and 20% (3/15) of DNA samples, respectively, whereas the rTth revealed PCR products in 80% (12/15) of FR3 and 60% (9/15) of FR2 reactions. Consistent clonal pattern was found in 67% (6/9) of FR3 and 33% (1/3) of FR2 PCR reactions with Taq, while the rTth used for detection presented consistent clonal pattern in 83% (10/12) of FR3 and 33% (3/9) of FR2 PCR analysis.

Conclusion: We conclude that the use of rTth polymerase, in combination with four template DNA dilutions, improves the sensitivity, specificity and reproducibility of the PCR-based IGH rearrangement analysis. This assay is sensitive, easy and fast for routine diagnostic applications, and we recommend it for small routinely processed tissue samples.

**068 THYMIDINE PHOSPHORYLASE (TP)-POSITIVE DENDRITIC CELLS (DC) CHARACTERIZE HISTOGENESIS OF GASTRIC B-CELL MALIGANT LYMPHOMAS (GBML) AND MAY EXPLAIN MECHANISM OF ANTIHELICOBACTER PYLORI (HP) THERAPY**

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A close relation between Helicobacter pylori (HP) infection and gastric B-cell malignant lymphomas (GBML) has been clarified and effective anti-HP therapy is established as the first choice of the GBML therapy. In order to see the mechanism of the anti-HP therapy, histogenesis of GBML was analyzed from a view point of stromal cells in 34 cases of GBML. Lymphocytes and stromal cells were labeled by ABC method and antibodies to CD3, CD20, CD68, CD34, anti-S100 protein, thymidine phosphorylase (TP) and inducible nitric oxide synthase (iNOS) in 11 cases of MALT type and 23 cases of DLBCL. Many TP-positive dendritic cells (DCs) intermingled and formed meshwork background in 8 cases of MALT type and 17 cases of DLBCL. Many CD68-positive stromal cells were seen in 9 cases of MALT type and 22 cases of DLBCL. A close relation between the TP-positive DCs and the CD68-positive cells was shown in each case. There were many intermingling CD3+ T-cells in most cases. In the germinal center (GC) colonization the stromal cells were positive for TP and iNOS in MALT type, but not in DLBCL. Thus, many CD68-positive macrophage-derived, T-cell-associated and TP-positive DCs characterized the histogenesis of GBML. This background of GBML may explain the mechanism of the anti-HP therapy because these stromal cells would be included in anti-HP therapy. The HP-induced stromal DCs may explain the transformation of GBML cells because of the nitric oxide-rich microenvironment in the GC colonization.
Materials/Methods: We evaluated the histological aspect of 24 cases of PLB in our institution (1988–2000). The immunophenotype used a large panel of monoclonal antibodies.

Results: The patients range in age from 28 to 80 years with male predominance 17/7. The tumour involved: temur (6), humerus (1), cubitus (1), clavicle (2), acromion (2), iliolum (5), bone vertebra (5), sacrum (1), and rib (1). The histological pattern was always diffuse, mostly composed of large cells. In the WHO classification, 10 were polymorphic centroblastic, 8 showed prominent multilobated cells, 2 were immunoblastic type. One was ALCL and 3 were diffuse large cells not otherwise specified. Sclerosis was an important feature in 2 cases, 23 cases were B cell type, CD20 CD79α+, 4 of those were also CD10+. Monotypic Ig expression was demonstrated in 11 cases, Bcl in 19. One was a T cell rich B lymphoma, one a null ALCL. LMP was negative in all cases. MDR expression was detected in variable amounts in 15 B cell tumours.

Conclusion: This work shows that as in previous reports, most PLBs are diffuse large B cell lymphomas. As mentioned by Harris in 1999, multilobated cells and important sclerosis were observed. We would like to underline some points of interest:

- 2 cases of rare pattern: one case of T cell rich B lymphoma, one ALCL rarely reported in bone (Medeiros 2000).
- No association with EBV LMP protein.
- No correlation between MDR expression either with relapse or with survival after treatment. All the patients received chemotherapy only, or chemotherapy associated with radiotherapy, without surgery.

**GENETIC ANALYSIS OF PRIMARY CUTANEOUS MALT-TYPE LYMPHOMAS: SOMATIC FAS MUTATIONS, BUT LACK OF BCL10 MUTATIONS AND THE t(11;18) TRANSLOCATION**

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GENetic alterations that allow tumor cells to evade apoptosis have recently been identified as key features of extracutaneous mucosa-associated lymphoid tissue (MALT)-type lymphomas. The t(11;18) translocation, which juxtaposes the anti-apoptotic API2 gene to the MALT1 gene, has been identified in a large proportion of MALT-type lymphomas, and a lower fraction of tumors harbor mutations that inactivate the pro-apoptotic functions of Fas and Bcl10. Here, we have examined the status of these genes in 19 primary cutaneous B-cell lymphomas (PCBL), 12 of which were MALT-type lymphomas. None of the cases carried the t(11;18) translocation, and tumor-specific Bcl10 alterations were not identified either at the genomic level or at the mRNA level. Somatic Fas mutations causing truncation of the Fas receptor were identified in two MALT-type lymphomas. One of the patients with Fas-mutated tumors exhibited autoimmune diabetes and rheumatoid arthritis, and the other had a 25-year history of benign cutaneous B-cell lymphocytoma. We hypothesize that Fas mutation may act equivalently to chronic antigen stimulation in the acquisition of cutaneous “MALT”.

**TRISOMY 3 AND GAIN OF AN X-CHROMOSOME ARE FREQUENT CYTOGENETIC ABNORMALITIES IN GASTRIC B-CELL LYMPHOMAS. ANALYSIS BY FISH ON TISSUE MICROARRAYS OF 226 SURGERICALLY RESECTED CASES**

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In the WHO classification Burkitt-like lymphoma is listed as a morphological variant of Burkitt’s lymphoma (BL), in addition to the three subcategories—endemic, non endemic, and immunodeficiency associated—proposed to reflect the major clinical and genetic subtypes of this disease. Different types of BL have been reviewed and studied by immunohistochemistry and molecular methods. Our results point out the heterogeneity of BL and suggest that AIDS related BL can have a different pathogenesis from classical endemic BL. The molecular heterogeneity of morphologically uniform diagnostic categories implies a need for molecular markers. In developmental biology a phenocopy is a mutation with a particular phenotype identical to that caused by a different genetic lesion. Many different molecular lesions which contribute to the disorders and behaviour of neoplastic cells and neoplasia with different profiles of molecular abnormality may be morphologically similar. That is, tumours of a given histological subtype may represent phenocopies, but need not behave alike because of the difference in molecular events that lead to their genesis and progression. This seems to be the case in BL.

**Burkitt’s lymphoma and lymphomas in immunocompromised patients**

L. Leoncini, S. Lazzi, C. Bellan, G. De Falco, A. Nyongo, A. Giordano, P. Tosi. Institute of Pathological Anatomy and Histology, University of Siena, Italy

In the WHO classification Burkitt-like lymphoma is listed as a morphological variant of Burkitt’s lymphoma (BL), in addition to the three subcategories—endemic, non endemic, and immunodeficiency associated—proposed to reflect the major clinical and genetic subtypes of this disease. Different types of BL have been reviewed and studied by immunohistochemistry and molecular methods. Our results point out the heterogeneity of BL and suggest that AIDS related BL can have a different pathogenesis from classical endemic BL. The molecular heterogeneity of morphologically uniform diagnostic categories implies a need for molecular markers. In developmental biology a phenocopy is a mutation with a particular phenotype identical to that caused by a different genetic lesion. Many different molecular lesions which contribute to the disorders and behaviour of neoplastic cells and neoplasia with different profiles of molecular abnormality may be morphologically similar. That is, tumours of a given histological subtype may represent phenocopies, but need not behave alike because of the difference in molecular events that lead to their genesis and progression. This seems to be the case in BL.

**MOLECULAR PATHOGENESIS**

L. Leoncini, S. Lazzi, C. Bellan, G. De Falco, A. Nyongo, A. Giordano, P. Tosi. Institute of Pathological Anatomy and Histology, University of Siena, Italy

In the WHO classification Burkitt-like lymphoma is listed as a morphological variant of Burkitt’s lymphoma (BL), in addition to the three subcategories—endemic, non endemic, and immunodeficiency associated—proposed to reflect the major clinical and genetic subtypes of this disease. Different types of BL have been reviewed and studied by immunohistochemistry and molecular methods. Our results point out the heterogeneity of BL and suggest that AIDS related BL can have a different pathogenesis from classical endemic BL. The molecular heterogeneity of morphologically uniform diagnostic categories implies a need for molecular markers. In developmental biology a phenocopy is a mutation with a particular phenotype identical to that caused by a different genetic lesion. Many different molecular lesions which contribute to the disorders and behaviour of neoplastic cells and neoplasia with different profiles of molecular abnormality may be morphologically similar. That is, tumours of a given histological subtype may represent phenocopies, but need not behave alike because of the difference in molecular events that lead to their genesis and progression. This seems to be the case in BL.

**ABSTRACT 71 TRISOMY RESULTS**

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**073** AGGRESSIVE B-CELL LYMPHOMAS IN IMMUNOCOMPROMISED PATIENTS, POST TRANSPLANT CASES

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The new WHO classification divides the post-transplant lymphoproliferative disorders (PTLD) into four major categories: early lesions (including reactive plasmacytic hyperplasia and infectious-mononucleosis-like cases), polyoma virus (MV), PTLD and Hodgkin lymphoma/Hodgkin lymphoma-like PTLD. The monomorphic PTLD are then categorized according to the standard lymphoma classification used for immunocompetent patients. The major B-cell M-PTLD include diffuse large B-cell lymphoma (DLBCL) and the less common Burkitt-like lymphoma (BL), plasmacytoma-like lesions and myeloma. The DLBCL-type PTLD include immunoblastic, centrolobulcent and occasionally anaplastic morphologic variants. Some of these cases are pleomorphic but, unlike classic polymorphic cases, demonstrate numerous transformed cells. Among the PTLD, these aggressive B-cell proliferations are associated with the most dominant B-cell clones; secondary genotypic abnormalities such as c-myc rearrangements or p53 mutations and are most likely to have bcl-2 mutations. The latter are also found in some PPTLD and are reported to occur in association with adverse prognostic factors. Studies leading to the conventional wisdom that PTLD develop from polyclonal often Epstein-Barr virus (EBV) driven lymphoid proliferations. An increasingly dominant clonal B-cell population then develops, followed by secondary genotypic abnormalities resulting in the type of PTLD that is unlikely to respond to decreased immunosuppression. Many admixed T-cells may be present particularly in the early lesions. Whereas PPTLD are well known to be very distinctive, aside from their EBV positivity, little is known as to how the M-PTLD of DLBCL type compare to the PTLD that arise in immune deficiencies or in immunocompetent patients. The presence of bcl-2 mutations might suggest a follicular center cell (FCC) origin. Analogous to how HIV-associated lymphomas and other DLBCL have been studied, we are investigating how the PPTLD in patients with HIV infection by analyzing a follicle center phenotype. Overall, a minority of B-cell PPTLD appear to have an FCC phenotype (bcl-6+, CD138−), whereas the majority have a post-follicular phenotype (bcl-6−, CD138+). Another major question is the pathogenesis of the increasingly common EBV-negative PTLD. About 20% of other PTLD are EBV negative in our institution with almost all of these cases occurring after 1990. Compared to EBV positive cases, they occur later, are more likely to be of monoclonal type and appear to have a higher incidence of an FCC phenotype (similar to the DLBCL we have studied in immunocompetent individuals). They may have bcl-2 gene rearrangements. Thus they appear even more like conventional B-cell lymphomas than EBV positive PTLD although some still respond to decreased immunosuppression. HHV-8 does not appear to be important, although rare HHV-8 positive, EBV-negative PPTLD have been reported.

**074** EMERGING PATHWAYS IN THE DEVELOPMENT OF HIV-ASSOCIATED LYMPHOMA

A Carbone. Division of Pathology, Centro di Riferimento Oncologico, A Carbone.

The clinicopathologic spectrum of AIDS-related non-Hodgkin’s lymphomas (AIDS-NHL) includes systemic lymphomas, primary central nervous system lymphomas (PCNSL), and two rare entities, namely primary effusion lymphoma (PEL) and plasmablastic lymphoma of the oral cavity (PB). The vast majority of systemic AIDS-NHL belongs to three high-grade B-cell lymphomas: Burkitt’s lymphoma (BL), immunoblastic lymphoma (BL) and large cell centroblastic lymphoma (LCI). The clinicopathologic heterogeneity of AIDS-NHL is correlated with the heterogeneity of the molecular lesions associated with these lymphomas. The molecular lesions associated with AIDS-BL involve activation of c-myc, inactivation of p53 and infection by Epstein-Barr virus (EBV). EBV is found in 40% of BL cases and 90% of ILC. Rearrangements of BCL-6 are detected in 20% AIDS-LCL. If EBV infected, BCL-6 expressing AIDS-LCL fail to express the LMP1 antigen. Conversely, AIDS-BL is characterized by absence of BCL-6 expression, absence of BCL-6 rearrangements, and frequent expression of LMP1. The marked degree of biological heterogeneity of AIDS-NHL is highlighted by their histogenetic differences, since AIDS-NHL are related to distinct B-cell subsets (i.e. germinal center or post-germinatal center B-cells). The phenotypic pattern of AIDS-BL, either systemic or primarily localized to the central nervous system, and AIDS-PEL, reflects post-SC B-cells in all cases. New information on the molecular pathways associated with different clinicopathologic categories of AIDS-NHL may serve as a point of attack for pathogenic-driven therapies. Moreover, a greater knowledge of other biologic features of these tumors may help to find out new potential targets for "intelligent therapies."
of >90%, classic MYC/IGH fusion, no BCL2/IGH fusion or immunoreactivity for BCL2, and positive staining for CD43, CD10, Bcl-6, and p53 (strong). BL and DBCL had median growth fractions of 66% and 67% respectively. Translocations were more common in BL than DBCL: MYC/IGH—37% vs 7%, BCL2/IGH—38% vs 22%, and both MYC/IGH and BCL2/IGH—10% vs 4%, respectively. BL and DBCL showed variable immunoreactivity for CD43 (60% vs 21%), Bcl-6 (74% vs 77%), p53 (23% vs 20%, strong), CD10 (60% vs 23%) and Bcl-2 (80% vs 74%). The results indicate that BL is a distinct biological entity characterized by a growth fraction of >90% and classic MYC/IGH fusion. No adult tumor was identified that had features identical to BL. The growth fractions of BL and DBCL are nearly identical, so this cannot be used as a diagnostic criteria for BL. BL and DBCL in adults form a continuum explaining the difficulty in generating reproducible criteria for the distinction. Furthermore, MYC/IGH fusion or BCL2/IGH fusion or both are both uncommon seen in BL than DBCL, suggesting that BL may be a more advanced form of DBCL that has acquired additional abnormalities such as translocation of MYC and/or BCL2.

**077 CYTOGENETIC AND CLINICOPATHOLOGIC FEATURES OF B-CELL LYMPHOMAS ASSOCIATED WITH THE BURKITT TRANSLOCATION (8;14) (q24;q32) OR ITS VARIANTS**

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Burkitt lymphoma is associated with the translocation (8;14) or its variants (t(8;2) and (t8;22). However, the latter has been reported in other B-cell lymphomas, mostly at relapse of low grade tumours.

The purpose of the present study was to investigate the cytogenetic and clinicopathologic features of 32 adult patients with B-cell lymphomas associated with (t8;14), (t2;8) or (t8;22) at presentation, as demon-strated by conventional cytogenetics performed in a single institution. Based on morphology and immunophenotype, the 32 cases were divided into 4 categories: (1) 9 cases were classified as Burkitt lymphomas with respect to morphology, and a CD10+, bcl-2− phenotype with virtually 100% of Ki67+ cells; most cases did not show chromosomal abnormalities in addition to Burkitt translocation; (2) 7 cases were diffuse large B cell lymphomas with a bcl-2+ (7/7), CD10+ (4/7) phenotype and numerous karyotypic abnormalities; (3) 7 were B-cell lymphomas with cytological features of progression from "low grade" (follicular mantle cell, B-CLL) lymphomas; all were bcl-2+ and had additional chromosomal alterations in relation to the "low grade" lymphoma; (4) 28 cases had "Burkitt-like" morphology features that were not considered as Burkitt lymphoma diagnosed by bcl-2− (9/9), CD10+ phenotype. Interestingly, 8 of them displayed several chromosomal alterations. Overall, 8 of the 23 "non-Burkitt" lymphomas disclosed a t(1;4;18) and/or a t(3;4) rearrangement, including 6 patients with simultaneous alterations involving myc, bcl-2 and bcl-6 regions.

This study shows that, in adult patients, Burkitt lymphomas account for only 30% of lymphomas bearing a "Burkitt" translocation at presentation. Morphology and cytogenetics strongly support a proportion of them are de novo transformation of low grade lymphomas. Further studies are needed to investigate the clinical relevance of the subgroup of lymphomas with "Burkitt-like" morphologic features which appears more closely related to diffuse large B cell lymphomas on the basis of their genetic and phenotypic features.

**078 ANALYSIS OF MYC TRANSLOCATION BREAKPOINTS BY FLUORESCENCE IN SITU HYBRIDIZATION ON ROUTINELY PROCESSED BURKITT’ S LYMPHOMA SAMPLES**

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Burkitt’s lymphoma (BL) is characterized by reciprocal 8q24 translocations which result in dysregulation of myc gene by juxtaposition with immunoglobulin regulatory sequences. Apart from its pathogenic relevance, this genetic lesion represents a molecular marker of the disease bearing diagnostic and clinical implication. The regular detection of myc breakpoints is hampered by technical problems including the distribution of the translocation breakpoints over the large (±1.6 Mb) genomic region.

We report the detection of myc translocation breakpoints by segregation fluorescence in situ hybridization (FISH) assay on a series of archival BL samples.

Two probe sets were selected based on reported translocation breakpoints in endemic and non-endemic BL. They consisted of alternatively labeled PACs or pair of cosmids, flanking the breakpoints regions from 600 kb 5' to 360 kb 3' of myc gene.

Both probe sets were validated in FISH segregation assay on 5 B-cell lines (Daudi, Ba/F3 and Nalp) and 31 lymphoma samples with cytogenetically proven 8q24 abnormalities.

In addition, routinely processed samples of 59 BL from Western Europe, South America and equatorial Africa were analyzed.

Myel translocation breakpoints were identified by FISH in 54 of 59 (92%) BL patients. In 48 (81%) the breakpoints were located in the genomic region from 50 kb downstream to 170 kb upstream. Five patients harbored breakpoints in more than 200 kb 3' and 5' of myc and in one patient an insertion of myc into IgH region was detected. All tumors expressed B-cell markers, had a high proliferative rate (Ki67 labeling index 95–100%) and were mostly CD10/bcl6 positive and bcl2 negative.

In conclusion the method allowed detection and analysis of myc translocation breakpoints in large series of Burkitt’s lymphoma in correlation with morphology, immunophenotype, EBV status, epidemiology and clinical presentation.

**079 EXPRESSION OF HPC2 AND HP H1 POLYCOMB GROUP PROTEINS NEXT TO BMI-1/RING1 SUGGESTS FUNCTIONALITY OF THIS COMPLEX IN HIGH GRADE B-NON HODGKIN LYMPHOMAS**

FJ van Kemenade, J Snijders, H Comajeta, GJ Kaspers, RJ Jonkhoff, M Jiwa, E Fieret, AP Otte, CJLM Meijer, FM Raaphorst. Dept of Pathology, VU-Medical Centre, Amsterdam, The Netherlands

**Introduction/Aim:** Polycromb-group (Pc-G) proteins are gene silencers that play an active role during embryogenesis, hematopoiesis and regulation of the cell cycle. Pc-G proteins form multimeric units, organ-izers that play an active role during embryogenesis, hematopoiesis and cell cycle. Intensity of staining was assessed semiquan-titatively.

In high grade B-NHL HPC1 and HPC2 could

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In conclusion the method allowed detection and analysis of myc translocation breakpoints in large series of Burkitt’s lymphoma in correlation with morphology, immunophenotype, EBV status, epidemiology and clinical presentation.

**Materials/Methods:** Burkitt’s and Burkittlike B-NHL lymphoma (N = 20) were selected from the pathology archive. All diagnoses were panel diagnoses. Clinical parameters, such as survival, response to chemotherapy, remission status, time of relapse, Karnofsky score, stage and IPI score were obtained from patient files. Expression of HPC-HPH complex Pc-G proteins [HPC1, HPH1, HPC2, BMI-1 and RING1] and the EED-EZH complex Pc-G protein EZH2 and BMI-1 were measured using immunohistochemistry, according to standard procedures. Intensity of staining was assessed semiquanti-tatively.

**Results/Conclusion:** In high grade B-NHL HPH1 and HPC2 could be detected in BMI-1+/EZH2+/BMI1+ blasts. HPC1 was only weakly expressed in a few cases. In contrast with normal dividing lymphoid cells, where there is no expression of BMI-1, RING1, HPH1 or HPC2, in BMI1+ tumour blasts these four Pc-G proteins can be detected. These findings suggest overexpression of these Pc-G genes in Burkitt’s and Burkittlike blasts and the possibility that functional HPC–HPH complexes are assembled in neoplastic cells. This may be relevant for Pc-G mediated gene inhibition in tumour cells of Burkitt’s lymphomas.
**080** THE INTERACTION BETWEEN HIV-1 TAT AND pRB2/p130: A POSSIBLE MECHANISM IN THE PATHOGENESIS OF AIDS-RELATED NEOPLASMS

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HIV-1 has long been recognized as the etiologic agent of acquired immunodeficiency syndrome (AIDS). Although many neoplasms arise in HIV-1 infected patients more frequently than in other forms of immunosuppression, the role of HIV-1 as an oncogenic virus has not yet been clarified. The HIV-1 gene product Tat, secreted by HIV-1 infected cells and taken up by normal cells, is a likely candidate to contribute to tumor pathogenesis in HIV-1 infected patients because of its growth promoting activity, angiogenic function and antiapoptotic effects. The oncogenic role of Tat is further supported by the development of non Hodgkin lymphomas in Tat-transgenic mice. Furthermore, we investigated whether Tat could influence either the phosphorylation state of pRB2/p130 or its expression at mRNA level. Preliminary results suggest that Tat may decrease the level of the hyperphosphorylated form of pRB2/p130, thus stabilizing the hypophosphorylated form of the protein. This may interfere with the ubiquitination pathway that controls pRB2/p130 levels in cells. The interaction between Tat and pRB2/p130 may lead to a deregulation of cell growth control by Rb-related proteins, that may contribute to lymphomagenesis in HIV-1 infected patients. The understanding of this basic information may be of significance for prognosis and implementing future therapeutic regimens, including the design of novel therapeutic approaches. As a matter of fact, spontaneous regression of HIV-1 associated lymphoproliferative disorders has been reported following highly active antiretroviral therapy.

**081** AIDS-RELATED LYMPHOMA SINCE THE ERA OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN A LOW SOCIO-ECONOMIC TERTIALLY FRENCH GROUP: HISTOLOGICAL TYPES AND EBV ASSOCIATION

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**082** HIV-RELATED LYMPHOMAS: CLINICOPATHOLOGICAL FEATURES

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Background: NHL and HL in HIV patients show similar histopathological characteristics as in non-seropositive patients, although they clinically differ in staging, diagnosis, treatment and outcome. HIV infection is a peculiar risk factor for NHL and HL. 70% of NHL and 30% of HL in HIV patients appear in the context of AIDS. Although many neoplasms arise in HIV infected patients more frequently than in other forms of immunodeficiency syndrome (AIDS). Although many neoplasms arise in HIV-infected patients more frequently than in other forms of immunosuppression, the role of HIV-1 as an oncogenic virus has not yet been clarified. The HIV-1 gene product Tat, secreted by HIV-1 infected cells and taken up by normal cells, is a likely candidate to contribute to tumor pathogenesis in HIV-1 infected patients because of its growth promoting activity, angiogenic function and antiapoptotic effects. The oncogenic role of Tat is further supported by the development of non Hodgkin lymphomas in Tat-transgenic mice. Furthermore, we investigated whether Tat could influence either the phosphorylation state of pRB2/p130 or its expression at mRNA level. Preliminary results suggest that Tat may decrease the level of the hyperphosphorylated form of pRB2/p130, thus stabilizing the hypophosphorylated form of the protein. This may interfere with the ubiquitination pathway that controls pRB2/p130 levels in cells. The interaction between Tat and pRB2/p130 may lead to a deregulation of cell growth control by Rb-related proteins, that may contribute to lymphomagenesis in HIV-1 infected patients. The understanding of this basic information may be of significance for prognosis and implementing future therapeutic regimens, including the design of novel therapeutic approaches. As a matter of fact, spontaneous regression of HIV-1 associated lymphoproliferative disorders has been reported following highly active antiretroviral therapy.

Results: Twenty-two patients, 17 males and 5 females (mean age, 39 years). Histological diagnoses were 14 NHL (6 DBCL, 2 primary effusion lymphoma, 3 Burkitt, 2 anaplastic lymphoma, 1 FL), and 8 HL (3 NS, 3 MC, 1 LD, 1LRCHL). Eight of NHL were extranodal lymphomas (2 primary effusion lymphoma, 2 oral cavity, 2 cutaneous, 1 cervical, 1 gastric). A marker of EBV detection by in situ hybridization technique, using the EBER probe. The association with EBV is present in 30% (Burkitt) to 100% (CNS lymphoma) of cases. Complete remission is about 50%. Prognostic factors in extracerebral lymphomas are IPI (most reliable indicator), CD4 <100, intravenous drug use, advanced stage and AIDS defining illness. Advanced stage was obtained in 70% of patients. Conclusions: In our series most of the lymphomas were high-grade NHL, involving extranodal sites in 8 cases. All HL were nodal. Most patients had normal levels of LDH. Malignant lymphoma was the first symptom in 75% of cases. Overall survival was 75%. Complete remission was obtained in 70% of patients.

**083** POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS (PTLD): MORPHOFUNCTIONAL AND HISTOGENETIC ANALYSIS OF 13 CASES

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Background: Post-transplantation lymphoproliferative disorders (PTLD) represent a clinical and histopathological heterogeneous
group of lymphoid proliferations of different clonal composition. Their occurrence varies according to the organ transplanted, type and degree of immunosuppressive therapy. PTLDs comprise a wide spectrum, from early, Epstein-Barr virus (EBV)-driven polyclonal proliferations to EBV- or EBV- lymphomas, mostly of B-cell origin.

**Purpose:** Among 765 heart or lung transplant recipients, treated and followed at the Cedars-Sinai Medical Center, Los Angeles, CA, USA, 12 cases of PTLDs were identified between B and BLL cases.

**Results:** The 12 cases of monomorphic PTLDs were classified according to the WHO lymphoma classification and consisted of 10 peripheral B-cell lymphomas (1 Burkitt lymphoma and 9 diffuse large B-cell type) and 2 peripheral T-cell lymphoma, anaplastic large cell lymphoma/AcL/TCL type (1 systemic and 1 primary cutaneous). Molecular analyses revealed a monoclonal rearrangement for IgH in 7/13 cases, whereas 6/13 had a “germline” configuration both for IgH and TCRα chain; a search for EBV by means of immunohistochemistry and/or in situ hybridization gave positive results in 7/13. The group of PT-B-cell lymphomas was also investigated for immunoglobulin variable (IgV) heavy (H) and light (L) chain gene mutational pattern and for a series of histogenetic markers (i.e. CD10; CD138; bcl-2; bcl-6; Maf and members of bcl-6 gene).

**Conclusion:** These investigations indicated that PT-related lymphomas are mostly of B cell origin and derive from GC-related B-cells, conceivably reflecting a post-GC stage of B-cell differentiation, which has not yet undergone preterminal maturation.

Molecular biology and molecular markers of aggressive B-cell lymphomas

**MOLECULAR PATHOGENESIS OF HUMAN B CELL LYMPHOMA**

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Non-Hodgkin lymphoma (NHL) derives from mature B cells (85% of cases), and, in a minority of cases, from T cells. Most B-NHL types derive from the germinal center (GC), the structure where naïve B cells encounter the antigen, undergo immunoglobulin (Ig) V region somatic hypermutation (SH) and isotype switching (S), and are selected to become memory B cells or plasma cells. SH and S mechanisms are involved in the generation of specific chromosomal translocations, which contribute to the pathogenesis of NHL by deregulating the expression of oncogenes like BCL2, c-MYC, BCL1, and BCL6. Recent progress will be presented in three areas: (i) analysis of the signaling pathways controlling normal and neoplastic GC formation by gene expression profiling; (ii) evidence that the somatic hypermutation mechanism is aberrantly activated in a subset of NHL to the targeting of ancestral CpG islands and, possibly, to the generation of chromosomal translocations; (iii) evidence that the function of BCL6, the transcription factor controlling GC formation and expressed in most B-NHL, is controlled by three distinct pathways which can be modulated for therapeutic purposes.

**MOLECULAR DIAGNOSIS AND PATHOGENESIS OF LYMPHOMA USING GENE EXPRESSION PROFILING**

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Genome-wide knowledge of gene expression in cancer cells promises to influence many aspects of cancer care. We are profiling gene expression in lymphoid malignancies using a specialized cDNA microarray, termed the “Lymphochip”, that is enriched in genes which are selectively expressed in lymphocytes and genes which regulate lymphocyte function. Gene expression profiling of diffuse large B-cell lymphoma (DLBCL) revealed that this single diagnosis actually contains two different diseases that differ in the expression of hundreds of genes (Alizadeh et al, Nature 2000;403:503). One DLBCL type strongly resembled normal normal germinal center B cells in gene expression and was therefore termed germinal center B-like (GCB) DLBCL. The other DLBCL type did not express the “signature” genes of germinal center B cells, but rather expressed genes that are induced in blood B cells upon mitogenic stimulation and was therefore termed activated B-like (ABC) DLBCL. Clinically, these DLBCL subgroups identified patients with distinct outcomes following chemotherapy.

An international consortium, termed the Lymphoma/Leukemia Molecular Profiling Project (LIMPP) has been formed to extend these observations in DLBCL and to use gene expression profiling to define molecular diagnostic categories of other lymphoid malignancies. This project is an expanded analysis of DLBCL continuing the existence of the GCB and ABC subgroups to demonstrate that additional DLBCL subgroups exist. GCB DLBCL patients had a relatively favorable prognosis compared to the other subgroups, but the DLBCL subgroup distinction did not capture the full extent of variability in survival of these patients following chemotherapy. Therefore, clinical data were used to discover individual genes and pathways that influence clinical outcome. Predictor genes were used to create a multivariate gene expression outcome predictor for DLBCL that stratified these patients into strikingly distinct risk groups. This biologically based outcome predictor can form the basis for more accurate diagnosis of DLBCL.

Gene expression profiling has also illuminated the molecular pathways responsible for the inferior clinical outcome of ABC DLBCL patients and has defined new molecular targets in this DLBCL subtype. The NF-κB signaling pathway was found to function constitutively in ABC DLBCL but not GCB DLBCL. This finding suggests that NF-κB is a new molecular target for ABC DLBCL patients. Further, the selective use of NF-κB pathway by ABC DLBCL reinforces the view that the two types of DLBCL defined by gene expression profiling are distinct diseases that rely upon different pathogenetic mechanisms.

**CLINICAL TRANSLATION OF MOLECULAR EVENTS: CELL CYCLE DEREGULATION IN LYMPHOMA AS A PARADIGM**

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Non-Hodgkin’s lymphoma is a convenient model for analysis of cell-cycle deregulation, since it includes several different diseases characterised by variable clinical course and specific molecular abnormalities. The effort to reveal the genetic alterations responsible for this variability in the clinical course and response to therapy is disclosing a scenario whereby bcl6, c-myc and other genes commonly deregulated by translocations, play a key role. Low-grade lymphomas, typically showing low level of bcl6 and c-myc expression, are characterised by molecular abnormalities not