REVIEW OF THE LITERATURE

New developments in the pathology of malignant lymphoma: a review of the literature published from May to July 2008

J. Han van Krieken

Introduction

The advancement of knowledge on lymphomas is at times bewildering. As shown below, the amount of new data is enormous, and this is only a selection of studies that are already or in the near future relevant for hematopathologists. I hope that the length of this overview does not scare off the readership, since there are many interesting studies presented from all over the world and from many different journals.

Biology of lymphoma

The focus of research in many areas of cancer is getting knowledge from the biology of cancer into clinical practice by developing targeted therapies in association with methods that identify patients that will benefit from these new approaches. The last few months' several studies indicate potential targets in lymphomas, and we will see in the next couple of years how this will lead to improved treatment.

Hodgkin lymphoma

The rosetting of CD4+CD26− T cells in classical Hodgkin's lymphoma (cHL) is a well-known phenomenon, but little is known about the gene expression profile and significance of these T cells. A higher percentage of CD4+CD26− T

J. H. van Krieken (✉)
Department of Pathology,
Radboud University Nijmegen Medical Centre,
P.O. Box 9101, 6500 HB Nijmegen, The Netherlands
e-mail: J.vanKrieken@pathol.umcn.nl

cells is present in nodular sclerosis Hodgkin lymphoma (NSHL) than in reactive lymph nodes. The resting CD4+ CD26− T cells in NSHL showed higher mRNA levels of CD25, CTLA4, OX40, and CCR4 compared with lymph nodes, supporting a regulatory T cell (Treg) type, and this was validated by immunohistochemistry. Moreover, these cells showed low or no expression of the Th1- or Th2-related cytokines IL-2, IFN-gamma, IL-13, IL-12B, IL-4, and IL-5 and the chemoattractant receptor CRTH2. Besides Tregs, Th17 cells may exist in NSHL based on the significantly higher IL-17 mRNA level for both T cell populations in NSHL. Upon stimulation in vitro, lack of upregulation of mRNA levels of most cytokine genes indicated an anergic character for the CD4+CD26− T cell subset. Anergy fits with the Treg profile of these cells, probably explaining the immunosuppressive mechanism involved in NSHL [1].

PRDM1/Blimp-1, a master regulator in terminal B cell differentiation, has been recently identified as a tumor suppressor target for mutational inactivation in diffuse large B cell lymphomas (DLBCL) of the activated B cell type. PRDM1/Blimp-1 is also a target for microRNA (miRNA)-mediated downregulation by miR-9 and let-7a in Hodgkin/Reed–Sternberg (HRS) cells of Hodgkin lymphoma (HL). These miRNAs target specific binding sites in the 3′ untranslated region of PRDM1/Blimp-1 mRNA and high levels of miR-9 and let-7a in HL cell lines correlated with low levels of PRDM1/Blimp-1. Similar to their in vitro counterparts, the majority of HRS cells in primary HL cases showed weak or no PRDM1/Blimp-1 expression. MiRNA-mediated downregulation of PRDM1/Blimp-1 may contribute to the phenotype maintenance and pathogenesis of HRS cells by interfering with normal B cell terminal differentiation, thus representing a novel molecular lesion, as well as a potential therapeutic target in HL [2].

Galectin-1 (Gal1) is an immunomodulatory glycan-binding protein regulated by an AP1-dependent enhancer in HRS cells and its expression affects the microenvironment in HL. Rodig et al. studied the expression of GAL1 and AP1 pathway proteins in 225 cases of various lymphomas and found that Gal1 is selectively expressed by Reed–Sternberg cells in >90% of primary cHLs and ALCL, in concordance with the activated AP1 component, c-*jun*. In contrast, DLBCL, primary mediastinal large B cell lymphoma, and another Hodgkin-related entity, nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), do not express Gal1 [3].

B cell lymphomas

In my previous review of the literature [4], I wrote that predictive markers are only limitedly available for lymphomas. This may soon change, since Yang at al. demonstrate that Src tyrosine kinase inhibition by dasatinib has antilymphoma effects because it inhibits cell growth in five of seven DLBCL cell lines. Compared to resting B cells, DLBCL has increased tyrosine phosphorylation activities. Dasatinib inhibits phosphorylation of several Src family kinase members, but in all cell lines regardless of their proliferative response to the drug. In contrast, the activity of two downstream signaling molecules, Syk and phospholipase Cgamma2 (PLCgamma2), are well-correlated with cell line sensitivity to dasatinib, suggesting that these molecules are crucial in mediating the proliferation of activated lymphoma cells. These findings show that dasatinib is a potentially useful therapy for DLBCL but also indicate that Syk and PLCgamma2 are potential biomarkers to predict dasatinib therapeutic response [5], a situation very similar to that of KRAS wild type predicting response to EGFR blocking in colorectal cancer.

Tissue inhibitor of metalloproteinase-1 (TIMP1) is a survival factor of germinal center (GC) B cells, and its overexpression is correlated with aggressive B cell lymphomas and cHL. TIMP1 downregulates B cell receptor and BCL6 and activates interleukin (IL)-6, IL-10/signal transducer, and activator of transcription-3 (STAT3) signaling in GC B cells. Kim et al. show that TIMP1 upregulates cell surface CD44 (standard and variants 3 and 7–10) and induces the activity and nuclear localization of SHP1 in an Epstein–Barr virus (EBV)-negative Burkitt lymphoma (BL) cell line, the neoplastic counterpart of GC centroblasts, suggesting that TIMP1 functions as a differentiating and survival factor of GC B cells by modulating CD44 and SHP1 in the late centrocyte/post-GC stage [6].

Increasing numbers of proteins are described that are involved in lymphomagenesis and influencing them might result in targeted therapies. Garrison et al. make this point for PUMA which encodes a BH3-only proapoptotic protein and which is targeted by p53. In a mouse model, cell lines, and cases of BL, it is shown that loss of PUMA frequently occurs, but that it can be reactivated by inhibition of DNA methyltransferases [7].

Nyagol et al. studied the role of the HIV-encoded Tat gene for inducing angiogenesis in HIV-associated lymphomas, since this may explain their aggressive behavior. Angiogenic switch marks the beginning of tumor's strategy to acquire independent blood supply. VEGF receptors are targeted by Tat protein from the HIV-1-infected cells due to the similarity of the basic region of Tat to the angiogenic factors (basic fibroblast growth factor, VEGF). VEGF promoter activity was downregulated in vitro in cells transfected with Tat. Reduced VEGF protein expression in primary HIV-1-positive BL and DLBCL, compared to the negative cases, supported the findings of promoter downregulation from the cell lines. Microvascular density assessed by CD34 expression was, however, higher in HIV-1-positive than in HIV-1-negative tumors. These results suggest that Tat has a wider angiogenic role, besides the regulation of VEGF expression. Thus, targeting Tat protein itself and stabilizing transient silencing of VEGF expression or use of monoclonal antibodies against their receptors in the AIDS-associated tumors will open a window for future explorable pathways in the management of angiogenic phenotypes in the AIDS-associated non-Hodgkin's lymphomas (NHL) [8].

Since recently developed drugs that target the antifibroblast growth factor receptor 3 (FGFR3) pathway are being tested in multiple myeloma in which about 15% cases carry the t(4;14) resulting in overexpression of FGFR3, Larson et al. analyzed 70 cases of malignant lymphoma. Only weak to moderate expression was found in 12% of cases, so it is unlikely that FGFR3 targeting is an effective treatment in lymphomas as well [9].

FOXP1 is targeted by chromosome translocations in mucosa-associated lymphoid tissue (MALT) lymphoma and DLBCL where high-level protein expression is associated with poor prognosis but the incidence and nature of FOXP1 abnormalities at both the genetic and protein levels and their correlation in these lymphomas are not well-established. Goatly et al. studied, by fluorescence in situ hybridization (FISH) and immunohistochemistry, a very large series ($n=321$) of extranodal marginal zone lymphomas and MALT lymphoma with a DLBCL component ($n=59$), nodal DLBCL ($n=64$), and extranodal DLBCL ($n=151$). FOXP1 translocation was found in eight MALT lymphomas and three MALT lymphomas with DLBCL with all positive cases originating in the stomach. In DLBCL, the translocation was seen in five cases originating in the stomach (two cases), tonsil (one case), large intestine (one case), and lymph node (one case). Three copies of the FOXP1 gene were observed in MALT lymphoma (17%),

MALT lymphoma with DLBCL (12%), and DLBCL (32%), including cases with FOXP1 translocation (19%). Immunohistochemistry showed strong/moderate FOXP1 staining in all the cases with FOXP1 translocation. However, FOXP1 expression was independent of FOXP1 translocation or copy number changes so that mechanisms other than translocation and copy number changes are also responsible for FOXP1 overexpression in lymphoma [10].

Functional characterization of signaling pathways that critically control mantle cell lymphoma (MCL) cell growth and survival is relevant to designing new therapies for this lymphoma. Hogan et al. analyzed the role of mdm2 aberrations in disrupting the p53 pathway in lymphomagenesis. At a physiological level, endogenous Pim-1 and Pim-2 interact with endogenous Mdm2 and the Pim kinases phosphorylate Mdm2 in vitro and in cultured cells at Ser (166) and Ser(186), two previously identified targets of other signaling pathways, including Akt. High levels of Pim expression, as occurs in tumors, active, but not inactive, Pim-1 or Pim-2 blocks the degradation of both p53 and Mdm2 in a manner that is independent of Mdm2 phosphorylation, leading to increased p53 levels and, proportionately, p53-dependent transactivation. Immunohistochemical analysis of a cohort of 33 human MCL show that elevated expression of Pim-1 occurs in 42% of cases with elevated Pim-2 occurring in 9% of cases, all of which also express Pim-1. Notably, elevated Pim-1 correlates with elevated Mdm2 in MCL. These data are consistent with the idea that Pim normally interacts with the p53 pathway but, when expressed at pathological levels, like in MCL, behaves as a classic dominant oncogene that stimulates a protective response through the induction of the p53 pathway [11]. Dal et al. demonstrate that the constitutive activation of Akt correlates with the expression of the phosphorylated, inactive form of PTEN. Phosphatidylinositol-3 kinase (PI3-K)/Akt or mammalian target of rapamycin (mTOR) inhibition decreased the growth of both primary MCL cultures and established cell lines and antagonized the growth-promoting activity of CD40 triggering and IL-4. These effects are mediated by nuclear accumulation of the p27(Kip1) inhibitor induced by the downregulation of the p45(Skp2) and Cks1 proteins, which target p27(Kip1) for degradation. Moreover, Akt inhibition downregulated cyclin D1 by promoting its proteasome-dependent degradation driven by GSK-3. Intriguingly, mTOR inhibition affected cyclin D1 proteolysis only in MCL cells in which GSK-3 is under the direct control of mTOR, suggesting that different MCL subsets could be differently responsive to mTOR inhibition. Finally, PI3-K/Akt inhibitors, but not rapamycin, induced variable levels of caspase-dependent apoptosis and reduced telomerase activity. These results indicate that Akt and mTOR activation have distinct functional relevance in MCL and suggest that

targeting Akt may result in more effective therapeutic effects compared with mTOR inhibition [12].

Wang et al. were able to create a mouse model for MCL that might help in further performing such studies. They isolated primary MCL cells from spleen, lymph node, bone marrow aspirates, or peripheral blood from six different patients and injected these into human bone chips, which had been implanted in SCID-hu. H&E staining and immunohistochemical staining with antihuman CD20 and cyclin D1 antibodies confirmed that patient's MCL cells were able to not only survive and propagate in the bone marrow microenvironment of the human fetal bone chips, but also similar to the human disease, migrate to the lymph nodes, spleen, bone marrow, and gastrointestinal tract of host mice. Treatment of MCL-bearing SCID-hu mice with atiprimod, a novel antitumor compound against the protection of bone marrow stromal cells, induced tumor regression [13].

As mentioned above, vascularization is crucial for the development of tumors and targeting tumor vessels has been shown to be effective in solid tumors of tumors. Giatromanolaki et al. investigated vascular density and the expression of hypoxia inducible factors (HIF) 1alpha and 2alpha, of vascular endothelial growth factor (VEGF), and of the phosphorylated form of its receptor VEGFR2 (pVEGFR2/KDR) in a series of 146 B cell NHL and 48 normal lymph nodes. Vascular density was significantly higher in the lymphoma compared to normal tissue, but there was no association with expression of the examined proteins. A significantly higher expression of HIF1alpha, VEGF, and pVEGFR2/KDR) was recorded in DLBCL compared to follicular lymphomas (FL). A strong statistical association of pVEGFR2/KDR expression with high HIF1alpha, HIF2alpha, and VEGF was noted in both DLBCL and FL. HIF1alpha andHIF2alpha were linked with VEGF expression, but no association between HIF1alpha and HIF2alpha was noted. It is concluded that the VEGF/receptor pathway is active in more than half of NHLs and particularly in DLBCL. The strong association of HIFalphas with the expression of VEGF and pVEGFR2/KDR found in the study provide strong evidence on the role of HIFalphas in the activation of angiogenic and VEGF-autocrine pathways that may be critical therapeutic targets for HIF inhibitors or other antiangiogenic agents [14].

T cell lymphoma

In T cell lymphomas, one may commonly find many eosinophilic granulocytes. Thielen et al. collected paraffin-embedded specimens from 50 patients diagnosed with peripheral T cell lymphomas, either unspecified (PTCL-U, $n=30$) or angioimmunoblastic (AITL, $n=20$). Morphological assessment for intratumoral eosinophilia and immunohistochemistry using specific antibodies directed against

TARC, IL-5, RANTES, and eotaxin showed that TARC- and IL-5-positive cases possessed significantly more eosinophils. Our data indicate that IL-5 and TARC expression highly correlate with eosinophilia in T cell lymphomas, suggesting that these chemokines are involved in the recruitment of eosinophils into the tumors [15].

Peripheral T cell lymphomas (PTCLs) are fatal in the majority of patients and novel treatments, such as protein tyrosine kinase (PTK) inhibition, are needed. The recent finding of SYK/ITK translocations in rare PTCLs led us to examine the expression of Syk PTK in 141 PTCLs. Syk was positive by immunohistochemistry (IHC) in 133 PTCLs (94%), whereas normal T cells were negative. Western blot on frozen tissue (n=6) and flow cytometry on cell suspensions (n=4) correlated with IHC results in paraffin. Additionally, Western blot demonstrated that Syk-positive PTCLs show tyrosine (525/526) phosphorylation, which is known to be required for Syk activation. FISH showed no SYK/ITK translocation in 86 cases. Overexpression of Syk, phosphorylation of its Y525/526 residues, and the availability of orally available Syk inhibitors suggest that Syk merits further evaluation as a candidate target for pharmacologic PTK inhibition in patients with PTCL [16].

Bonzheim et al. investigated single nucleotide polymorphisms (SNPs) of the FAS and CTLA-4 genes in 94 peripheral T cell lymphomas since tumor cells of these lymphoma fail to undergo apoptosis even in cases with the phenotype of effector T cells and high expression of FAS and CTLA-4 receptor molecules. Although allelic frequencies of some FAS SNPs were enriched in AILT cases, none of these occurred at a different frequency compared to healthy individuals. Therefore, SNPs in these genes are not associated with the apoptotic defect and autoimmune phenomena in peripheral T cell lymphoma [17].

Anaplastic large cell lymphoma (ALCL), ALK1-positive, is characterized by the constitutive activation of STAT3. Bard et al. describe the existence of an autocrine stimulatory loop involving interleukin-22 (IL-22) in ALCL by studying cases and cell lines. The IL-22 receptor, a heterodimer composed of IL-22R1 and IL-10R2, was expressed in all ALK(+)ALCL cell lines and tumors examined. The expression of IL-22R1 in ALK(+)ALCL is aberrant, as this protein is absent in benign lymphocytes. Although ALK(+)ALCL cells produce endogenous IL-22, the addition of recombinant IL-22 to ALK(+)ALCL cell lines significantly increased STAT3 activation, cell proliferation, and colony formation in soft agar. Nucleophosmin (NPM)-ALK, the characteristic fusion gene oncoprotein expressed in ALK(+) ALCL, directly contributes to the aberrant expression of IL-22R1, as transfection of NPM-ALK in Jurkat cells induced

IL-22R1 expression and IL-22-mediated STAT3 activation [18].

To identify highly recurrent genetic alterations typical of Sézary syndrome (Sz) high-resolution array-based comparative genomic hybridization was done on malignant T cells from 20 patients. Minimal common regions with copy number alteration occurring in at least 35% of patients harbored 15 bona fide oncogenes and three tumor suppressor genes. Based on the function of the identified oncogenes and tumor suppressor genes, at least three molecular mechanisms are relevant in the pathogenesis of Sz. First, gain of cMYC and loss of cMYC antagonists (MXI1 and MNT) were observed in 75% and 40% to 55% of patients, respectively, which were frequently associated with deregulated gene expression. Second, a region containing TP53 and genome maintenance genes (RPA1/HIC1) was lost in the majority of patients. Third, the interleukin-2 (IL-2) pathway was affected by gain of STAT3/STAT5 and IL-2 (receptor) genes in 75% and 30%, respectively, and loss of TCF8 and DUSP5 in at least 45% of patients. [19].

The cutaneous CD30-positive lymphoproliferations encompass a spectrum of disorders that share histological and phenotypic similarities but differ markedly in clinical behavior. Gjeerdrum et al. studied the expression of FoxP3 as a marker of Tregs in skin biopsies from lymphomatoid papulosis (LyP) (n=14), primary cutaneous anaplastic large cells lymphoma (C-ALCL) (n=13), and systemic anaplastic large cells lymphoma (S-ALCL) with (n=9) or without (n= 6) ALK expression. Only one case of C-ALCL was positive for FoxP3, and in three cases, occasional tumor cells were positive. All biopsies contained tumor-infiltrating FOXP3-positive Tregs, however, with significant higher numbers in ALK-negative S-ALCL and LyP than in C-ALCL and S-ALCL positive for ALK [20].

Epidemiology of lymphoma

Increasingly, reports from different regions in the world indicate the great variation in the epidemiology of cancer and many important lessons can be learned from that. Also, it is becoming clear that survival of patients with a variety of tumor types is really improving.

HL shows a bimodal distribution with a first peak in developing countries during childhood. The causative role and prognostic significance of EBV association in patients with HL is controversial. In two Latin-American pediatric HL series, EBV-encoded RNAs in situ hybridization and latent membrane protein 1 immunohistochemistry on HL biopsies from 176 pediatric patients was positive in 52% of

cases. EBV was significantly associated with mixed cellularity subtype but was not related to survival. The results do not support EBV association stated for pediatric HL in developing countries [21].

NHL is the most common hematologic malignant neoplasm in adults. The most recent trends in survival among adults diagnosed as having NHL on the population level in the US show that, overall, 5-year relative survival increased from 50.4% to 66.8% and 10-year relative survival increased from 39.4% to 56.3% between 1990–1992 and 2002–2004. Improvements were most pronounced in patients younger than 45 years (+26.8 and +27.1 percentage points for 5- and 10-year survival, respectively), but improvements were seen in all age groups, in both sexes, in both nodal and extranodal disease, and in both low-grade and high-grade disease. Improvements in prognosis were less in black patients than in white patients, especially in younger black patients. Changes in treatment of the disease and a decrease in the number of human immunodeficiency virus-related NHL cases attributable to highly active antiretroviral therapy are probably primarily responsible for these improvements [22].

The incidence, risk factors, and endoscopic presentation of gastrointestinal non-Hodgkin's lymphoma (GI NHL) was described in a large predominantly urban adult population sample by Andrews et al. Data over a 5-year period (1999–2003) from Calgary, Canada were collected and supplemented with data population-based HIV database (1985–2004). The 56 GI NHL cases imply an annual incidence of 1.73 per 100,000, the majority being DLBCL (54%), followed by extranodal marginal zone lymphoma (MALT lymphoma; 29%). Increasing age, history of kidney transplant, and *Helicobacter pylori* positivity in MALT lymphoma were identified as risk factors. Within the HIV-positive population, a highly significant drop in GI NHL was seen over time with an incidence of 3.86 per 1,000 patient-years in 1985–1989 compared to zero cases in 2000–2004, despite a greater prevalence of HIV disease [23].

Mwakigonja et al. were able to study 176 lymphoma cases from Tanzania, collected from 1996–2001. The proportion of lymphomas out of all diagnosed tumors was 4.2% (176/4,200) comprising 77.8% NHL including 19.3% Burkitt's (BL) and 22.2% HL and 23.7% of tested sera from these patients were HIV-positive. In this short period of time, malignant lymphomas increased significantly, predominantly among the young, HIV-infected, and AIDS patients [24].

The most common NHLs in Uganda are neoplasms of B cell derivation and 129 such cases were classified as BL (95 cases), DLBCL (19 cases), MCL (four cases), and B cell lymphoblastic lymphoma (one case). In BL, a homoge-neous phenotype (CD10(+), BCL6(+), Bcl-2(−), MUM1/IRF4−, and Ki-67 approximately 100%) and a stable EBV integration were found. A distinctive and unusual feature was the frequent plasma cellular differentiation, along with the positivity for CD30 and CD138 (recorded in 35 and 43 cases, respectively) [25].

EBV-associated lymphoproliferations are common in East Asia. Cho et al. determined the spectrum of EBV-positive lymphoproliferative diseases (LPD) and relationships between these diseases in Korea. The EBV status and clinicopathology of 764 patients, including acute EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH), chronic active EBV (CAEBV) infections, B-LPD arising in chronic latent EBV infection, T and natural killer (NK) cell NHL, B-NHLs, and HL were analyzed. T or NK cell NHLs were the most common forms of EBV-positive NHLs (107/167, 64%); among these, nasal type NK/T cell lymphomas were the most common (89/107, 83%). According to age, BL was the most common in early childhood; in teenagers, chronic (active) EBV infection-associated LPD was the most common type. The incidence of NK/T cell lymphoma began to increase from the twenties and formed the major type of EBV-associated tumor throughout life. DLBCL formed the major type in the elderly. In conclusion, primary infections in early childhood are complicated by the development of CAEBV infections that are main predisposing factors for EBV-associated T or NK cell malignancies in young adults. In old patients, decreased immunity associated with old age and environmental cofactors may provoke the development of peripheral T cell lymphoma, unspecified, and DLBCL [26].

Sjo et al. analyzed the epidemiology of the rare ophthalmic lymphoma in Denmark during the period 1980 to 2005. A total of 228 patients with a histologically verified diagnosis of ophthalmic lymphoma were included. There was an equal distribution of men and women. The most frequent lymphoma subtype was extranodal marginal zone B cell lymphoma (MALT lymphoma, 55.5%) and most cases were located in the orbit (56.8%). High-grade lymphoma subtypes were found more frequently in men than in women. Incidence rates were highly dependent of patient age. For all ages, a statistically significant annual average increase of 3.4% during the 26-year period was found. This was primarily due to a rise in incidence of MALT lymphoma [27].

Defining entities

Soon, the new WHO classification will become available. It is increasingly difficult to find a balance between lumping

and splitting since so much more information on individual cases becomes available. At the same time, for some entities, it remains difficult to classify them reliably. It is, however, difficult to make choices on which new tools need to be implemented in routine practice.

Hodgkin lymphoma

Programmed death 1 (PD-1) is a lymphoid receptor that negatively regulates immune responses and is a marker of follicular T cells that forms rosettes around the neoplastic cells in NLPHL. Nam-Cha et al. analyzed the diagnostic value of PD-1 staining in 152 cases of various types of HL and T cell-rich diffuse large B cell lymphoma (TRBCL). Results show that PD-1 (NAT-105) is a marker not only of follicular T cell rosettes in NLPHL, but also of a subset of lymphocyte-rich cHL and is absent in TRBCL. The presence of PD-1-positive T cell rosettes, therefore, seems to be an additional useful feature in the sometimes difficult differential diagnosis of NLPHL and T/HRBCL; in borderline cases (probably discussed in Bordeaux!), PD-1 positive rosettes are present, so these cases more likely represent NLPHL [28]. PD-1 expression was also reported in some T cell NHL subtypes. Xerri et al. describe the expression profile of PD-1 and its ligands (PD-L1 and PD-L2) in 161 lymphoma tissue and 11 blood samples of B-NHLs. In reactive lymph nodes, PD-1 was mainly expressed in follicular T cells. In B-NHLs, PD-1 was mainly expressed in reactive T cells as well, but expression was also noted in neoplastic B cells from small lymphocytic lymphoma (SLL, 12/13), grade III follicular lymphoma (3/3), and DLBL (2/25). In contrast, neoplastic B cells from MCL (0/11), marginal zone lymphoma (0/12), BL (0/3), and grade 1 to 2 follicular lymphoma (0/40) were PD-1 negative. PD-L1 and PD-L2 were negative in small B cell lymphomas, including B-SLL. Flow cytometry showed that blood cells from chronic lymphocytic leukemia (B-CLL) also displayed PD-1 expression, which could be increased by CD40 stimulation. PD-1 expression in T-NHLs was restricted to the angioimmunoblastic subtype (8/8). These results show that PD-1 expression among B-NHLs is mainly associated with SLL/CLL and is influenced by the activation of the CD40/CD40L pathway. Because the anti-PD-1.6.4 antibody works on paraffin sections, it represents a useful tool to differentiate SLL/CLL from other small B cell lymphomas [29].

B cell lymphomas

The WHO classification defines entities by morphology, immunophenotype, genetics, and clinical features. An important example of the latter is age, and it is increasingly recognized that pediatric lymphomas might be different from adult cases, even when they are the same subtype. Klapper et al. contradict this by analyzing 65 cases of mature aggressive B cell NHL from patients up to 18 years of age and compared to results to those of 182 adult patients. Gene expression profiling reclassified 31% of morphologically defined DLBCLs as molecular Burkitt lymphoma (mBL), but the subgroups obtained by molecular reclassification did not show any difference in outcome. No differences were detectable between pediatric and adult mBL with regard to gene expression or chromosomal imbalances. They conclude that based on molecular profiling mBL is a molecularly homogeneous disease across children and adults [30]. The important question remains whether molecular profiling will override the present criteria in classifying lymphomas of children; at least in this study, prognostic information based on standard diagnosis, i.e., DLBCL vs. BL gets lost after classifying according expression profiling.

As written in the previous review of the literature, follicular lymphoma in Japan is genetically different from cases in the west. Karube et al. studied 100 BCL2 rearrangement negative follicular lymphomas and identified four subgroups. Group I: BCL6 gene rearrangement ($n=$ 41); group II: BCL6 gene amplification/3q27 gain ($n=$30); group III: the absence of both ($n=$23); and group IV: the presence of both ($n=$6). Group II showed higher grade morphology (grade 3a/b, 93%), higher bcl2 and MUM1 expression (73 and 57%, respectively), and more frequent combination with BCL2 gene amplification/18q21 gain (90%) than the other groups [31]. Further studies are needed on the relevance of these findings.

In the Kiel classification, follicular centroblastic lymphoma was separated from follicular centroblastic centrocytic lymphoma and put in the category of centroblastic lymphoma. Piccaluga et al. showed that this separation has indeed a molecular basis, although both types belong to the same category and are distinct from other large cell lymphomas. Using expression profiling of 43 follicular lymphomas, 50 B cell NHLs of different type, and 20 samples of normal B lymphocytes, it became clear that the molecular profile of follicular lymphoma is intimately related to that of normal germinal center B cells, irrespective of the histological grade. Furthermore, follicular lymphoma has a relatively homogeneous gene expression profile that is distinct from that of other B cell NHL and does not include discrete molecular subgroups. However, by further clustering, grade I–IIIa tumors tend to cluster together, while grade IIIb follicular lymphoma constitutes a distinct subgroup whose molecular signature is closer to that of the remaining follicular lymphomas than to that of DLBCL of the germinal center B cell type [32].

Martinez et al. studied the expression of interferon regulatory factor (IRF) 8 in reactive lymphoid tissues and

in a series of 232 B cell tumors and 30 cell lines representing a variety of B cell developmental stages. Although IRF8 was detectable in most reactive B cells, its expression levels differed with developmental stage. Germinal center B cells contained the highest levels of IRF8 with lower levels seen in mantle and marginal zone B cells and none in plasma cells. IRF8 was coexpressed with PAX-5, Pu.1, and BCL 6 and similar to BCL6, was absent from the small population of IRF4-positive germinal center B cells thought to be committed to postgerminal center developmental programs. Similarly, IRF8 was most strongly expressed in lymphomas of germinal center origin with lower levels present in MCL, CLL, and marginal zone lymphomas, and no expression observed in plasmacytic/plasmablastic neoplasms. The reciprocal expression pattern with IRF4 in reactive tissues was generally maintained in lymphomas with some exceptions. These results provide a new diagnostic marker helpful in distinguishing B cell NHL subtypes [33]. Obviously, it will be important to know whether this marker may separate activated from germinal center DLBCL (see below, prognostic factors). Baecklund et al. describe another new germinal center marker, human germinal center-associated lymphoma protein (HGAL), in DLBCL of patients with rheumatoid arthritis. Of 111 cases, 38 (34%) DLBCL were HGAL-positive and showed less disseminated disease and a tendency toward improved overall survival compared to HGAL-negative cases. This supports that a majority of RA-DLBCL are of non-GC origin, indicating a specific role for activated peripheral B cells in the pathogenesis of RA-DLBCL [34].

The chemokines (CKs) CXCL13, CCL21, and CXCL12 are known to play differential roles in the organization of the lymphoid tissues and the development of lymphoid malignancies. Barone et al. showed that, within salivary glands with lymphoepithelial sialadenitis (LESA) and MALT lymphoma, the lymphoid CKs CXCL13 and CCL21 are selectively associated with areas of reactive lymphoid proliferation, whereas no significant expression of these molecules was detected in the malignant lymphoid aggregate. Conversely, CXCL12 was observed predominantly in infiltrated ducts and malignant B cells. Accordingly, CXCL13 and CCL21 transcript levels were significantly increased in LESA samples while CXCL12 levels were increased in MALT lymphoma and isolated tumor cells. Low levels of CK receptors were detected on lymphoma-extracted lymphocytes, suggesting downregulation in the abundance of ligands [35]. These findings may assist in the sometimes difficult distinction of reactive vs. malignant infiltrates in salivary glands, especially those of patents with Sögrens syndrome.

Nakamura et al. investigated the incidence and clinical significance of lymphoma-associated chromosomal translocations, particularly those involving the immunoglobulin heavy chain (IGH) gene locus, in a large series of gastric DLBCL (141 cases of primary gastric DLBCL [58 with MALT lymphoma and 83 without MALT lymphoma]) using FISH. Translocations involving IGH were detected in 36 (32%) of 111 cases; their partner genes included BCL6 ($n=10$), c-*myc* ($n=5$), and FOXP1 ($n=3$) but remained unknown in the remaining 18 cases. t(14;18)/ IGH-BCL2, t(14;18)/IGH-MALT1, and t(1;14)/BCL10-IGH were not detected in any case. t(11;18)/API2-MALT1 was detected in none of the cases, except for one case of DLBCL with MALT lymphoma, which showed positive signals only in MALT lymphoma cells. IGH-involved translocation was associated with younger age but not with any other clinicopathologic factors including GCB or non-GCB immunophenotypes. Cox multivariate analysis revealed that IGH-involved translocation, in addition to younger age and early stage, was an independent prognostic factor for better overall and event free survival [36].

The role of microRNAs is becoming increasingly clear. Several miRNAs have been reported to be associated with IgH mutation and ZAP-70 expression status in blood samples of B cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL). Wang et al. collected and analyzed 33 lymph nodes and 37 blood CLL samples and analyzed IgH mutation status, ZAP-70 expression status and expression of 15 miRNAs. Sixty-three percent of the lymph node cases contained mutated IgH genes and 49% of the lymph node cases were ZAP-70-positive, and a significant correlation was observed between ZAP-70 expression and IgH mutation status. Of the blood CLL samples, 49% contained mutated IgH sequences. The miRNA expression pattern in CLL lymph node and blood samples was very similar. Three of 15 miRNAs (miR-16, miR-21, and miR-150) showed a high expression level in both blood and lymph node samples. No difference was observed between ZAP-70-positive and ZAP-70-negative and between IgH-mutated and unmutated cases. No correlation was found between miR-15a and miR-16 expression levels and 13q14 deletion in the blood CLL samples [37].

In a series of 63 primary DLBCLs of bone collected from centers in France and Brazil, a substantial number of cases had a rearrangement of BCL2 (9/32) or c-*myc* ($n=3$), whereas the PAX5, BCL6, BCL1 cyclin D1, and ALK genes were in germline configuration. The majority of the cases with rearrangements were of the GC phenotype. These results, associated with the lack of BCL6 rearrangement, suggest that bone DLBCL represents a specific group within extranodal B cell lymphomas [38].

Primary lymphomas of the CNS (PCNSLs) show molecular features of the late germinal center exit B cell phenotype and are impaired in their terminal differentiation as indicated by a lack of immunoglobulin class switching. Because the positive regulatory domain I protein with ZNF

domain (PRDM1/BLIMP1) is a master regulator of terminal B cell differentiation into plasma cells, Courts et al. investigated a series of 21 PCNSLs for the presence of mutations in the PRDM1 gene and found deleterious mutations in four of 21 (19%) PCNSLs. They conclude that, similar to systemic DLBCLs, PRDM1 may be a tumor suppressor in some PCNSL and contribute to lymphomagenesis by impairing terminal differentiation [39]. Maybe as important, this finding goes against the notion that this PCNSL is a distinct entity.

Plasmablastic lymphoma (PBL) and multiple myeloma (MM) are B cell-derived malignancies that share many morphologic and immunophenotypic traits, making the differential diagnosis particularly complicated. Damasi et al., after demonstrating that peroxiredoxin I (PrdxI) is expressed in plasma cells but not in B lymphocytes, analyzed eight cases of PBL and nine of MM by immunohistochemistry. The results show that that PrdxI expression is closely connected with the immunoglobulin production capacity of the cells, which means high in MM, but absent in PBL cases, except one, wherein few cells were stained. PrdxI, therefore, could be considered an additional plasma cell functional marker and thus may serve as a marker for MM [40].

T cell lymphomas

Many years after his retirement, Prof. Lennert remains active in the field of lymphoma diagnosis. In a recent paper from the Kiel group, the importance of morphology is stressed in this era of expression profiling. Reevaluating a historical series of 17 lymphomas, diagnosed as lymphohistiocytic lymphoma according to the criteria of the Kiel classification with the presence of large purple macrophages (LPM) as the decisive finding for diagnosing this lymphoma subtype, the relation to the lymphohistiocytic variant of ALCL according to the WHO classification was studied. All cases in the cohort matched the criteria of ALCL according to the WHO classification; in 30% of the cases, the total amount of macrophages did not exceed the number of CD30-positive tumor cells. The results indicate that the presence of LPM might be helpful to identify this subgroup of ALCL. Because the distinction of morphological subtypes of ALCL is of clinical relevance, improved criteria for subtyping ALCL are urgently needed and might include the presence LPM as one such criterion [41].

Completely opposite to the conclusions by Verbeek et al. (see previous review of the literature in issue 1), celiac disease (CD) is characterized by villous atrophy and an increase in intraepithelial lymphocytes (IEL), of which the IEL usually exhibit a suppressor/cytotoxic phenotype (CD3 and CD8) and display a polyclonal profile for T cell receptor (TCR) rearrangement as opposed to the mono-

clonality of refractory CD (RCD) with CD8 IEL. A complication of CD is the loss of response to a gluten-free diet called RCD that may progress to an enteropathy-associated T cell lymphoma. De Mascarel et al. studied 20 uncomplicated CD and 23 complicated CD (19 RCD and four diagnosed at the same time as enteropathy-associated T cell lymphoma). In complicated CD, the IEL phenotype was CD8 in nine cases and CD8 in 14 cases. In 100% of cases, IEL showed a monoclonal TCR rearrangement. All the nine CD8 complicated CD exhibited a monoclonal TCR rearrangement and three of them were associated with a T cell lymphoma (two at the same time as CD and one after 43 months follow-up) and bore the same monoclonal rearrangement in IEL and in lymphoma. Interestingly, the 13 cases (100%) of CD with a CD8 phenotype were also found monoclonal and two of them were associated with a T cell lymphoma diagnosed at the same time as CD and exhibiting the same rearrangement in IEL and in lymphoma. An aberrant CD3 CD8 IEL phenotype is a good criterion for RCD diagnosis. However, cases with a normal CD3 CD8 IEL phenotype may correspond to RCD. In such cases, molecular analysis of TCR-gamma genes is a useful method for identifying cases with RCD [42]. It is unclear why the results of the two studies are so divergent.

Cutaneous lymphomas

The series of studies of the Willemze group on the classification of cutaneous lymphomas have firmly defined the existence of two distinct primary cutaneous large B cell lymphomas: primary cutaneous follicle center lymphoma (PCFCL), characterized by an excellent prognosis, and primary cutaneous large B cell lymphoma, leg type (PCLBCL leg type) with an unfavorable prognosis. Now van Galen et al. investigated 21 cases with expression profiling and conclude that the results suggest that the clinically favorable PCFCLs are characterized by a relatively intense cellular cytotoxic immune response and that PCLBCL leg types are characterized by constitutive activation of the intrinsic mediated apoptosis pathway with concomitant downstream inhibition of this apoptosis pathway [43].

New entities/subtypes

DLBCL usually proliferates effacing lymph follicles. In occasional cases, tumor cells show an interfollicular pattern of proliferation preserving lymph follicles. Yamauchi et al. showed that DLBCL with an interfollicular pattern of proliferation has distinct clinical and pathological findings compared to ordinary DLBCL. Twelve cases of DLBCL with an interfollicular pattern of proliferation [interfollicular

group (IF)] were compared to 30 cases of DLBCL with ordinary morphology [control group (CG)]. IF showed a significantly lower lactate dehydrogenase level and International Prognostic Index scores and the frequency of localized disease were higher than that in CG. Immunohistochemically, the majority (11 of 12) of IF cases showed a nongerminal center B cell phenotype which was higher than that in CG [44].

Most cases of intravascular large cell lymphoma have a B cell phenotype, but rare T cell and NK cell variants have been reported. Four patients (M/F=3:1; age range, 63 to 87 years; median age, 65 years) with intravascular large NK/T cell lymphoma were described by Cerroni et al. The skin was the site of presentation in all patients (leg, one case; trunk, one case; trunk and extremities, two cases). Two patients had lesions confined to the skin; in one case, concomitant involvement of the brain was detected, and in one case, no further studies were carried out. Immunohistology showed positivity for cytotoxic markers in three of four cases. One case had an NK phenotype similar to NK/T cell lymphoma, nasal type, whereas the other cases could not be precisely classified into specific categories. One of these cases was negative for cytotoxic markers and was positive only for CD2 and CD3. Association with EBV was demonstrated in two cases by in situ hybridization, whereas one case was negative. All the patients had aggressive disease and died between 2 weeks and 7 months from presentation. Intravascular large NK/T cell lymphoma is a rare, aggressive lymphoma with variable phenotypic features, frequent expression of cytotoxic proteins, true NK cell phenotype and association with EBV infection, and common presentation in the skin [45].

Nowadays, many reports deal with the new syndrome/entity IgG4-related sclerosing disease, which is a recently recognized inflammatory lesion frequently involving the pancreas, submandibular gland, lacrimal gland, and lymph node. Cheuk et al. describe the hematopathological features in this syndrome in two papers. The first one deals with the morphologic features of the enlarged regional (*n*=3) and nonregional lymph nodes (*n*=3) in patients. The histologic features of the lymph nodes could be categorized into three patterns: Castleman disease-like, follicular hyperplasia, and interfollicular expansion by immunoblasts and plasma cells. The percentage of IgG4+/IgG+ plasma cells was markedly elevated (mean 62% vs. 9.9% in 54 control lymph nodes comprising a wide variety of reactive conditions). Six cases of primary lymphadenopathy were characterized by increased IgG4+/IgG+ plasma cells (mean 58%). These cases share many clinical and pathologic similarities with IgG4-related sclerosing disease. In fact, two of these patients developed lymphoplasmacytic sclerosing cholangitis or lacrimal and submandibular gland involvement during the clinical course. These cases, therefore, probably represent

primary lymph node manifestation of the disease. The importance of recognition of the lymphadenopathic form of IgG4-related sclerosing disease lies in the remarkable response to steroid therapy, and the potential of mistaking the disease for lymphoma either clinically or histologically [46]. The second paper describes three cases of ocular adnexal lymphoma arising in IgG4-related chronic sclerosing dacryoadenitis. The patients presented with bilateral or unilateral ocular adnexal mass usually present for many years. One patient also had asymptomatic diffuse lymphadenopathy. Two patients had biopsy-proven IgG4-related chronic sclerosing dacryoadenitis before the current presentation, and one had systemic involvement by IgG4-related sclerosing disease as evidenced by increased IgG4+ cells in a prior nasopharyngeal biopsy. Two cases showed features of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type (one with large cell transformation) and one follicular lymphoma. Presently, the mechanism and impact of the findings remain unclear [47]. Kojima et al. describe IgG4 elevated serum levels in one of their three patients with reactive follicular hyperplasia and prominent interfollicular plasmacytosis in regional lymph nodes of their Küttner's tumor. The polytypic nature of plasma cells was demonstrated by immunohistochemistry. There were numerous IgG-positive plasma cells with scattered IgA-positive or IgM-positive plasma cells. IgG4-positive cells comprised 25% to 40% of IgG-positive plasma cells. These three cases indicated that regional lymph node of Küttner's tumor may show reactive follicular hyperplasia and prominent interfollicular plasmacytosis and should be differentiated from various benign and malignant lymphoproliferative disorders including systemic rheumatic disease, plasma cell type of Castleman disease, and lymph node involvement of marginal B cell lymphoma of the MALT type showing prominent plasma cell differentiation [48].

Pitfalls in lymphoma diagnosis

Farris et al. studied 11 cases of rectal tonsil (RT), a localized reactive proliferation of lymphoid tissue occurring in the rectum, which, since it is not well-known to many pathologists, can cause diagnostic difficulty, and awareness of this entity can prevent a misdiagnosis of lymphoma. The patients (six males and five females) were middle-aged adults, except for one case affecting a young boy (age range, 1 to 62 years; mean, 49 years). All presented with either rectal bleeding or abdominal pain or had the lesion found on routine screening. Histologically, all cases were composed of a lymphoid proliferation involving the lamina propria or submucosa. Lymphoid follicles could be identified in all cases, although some were difficult to appreciate

without immunostains for follicular dendritic cells. Intraepithelial lymphocytes were present in nine cases, and five cases showed nondestructive lymphoepithelial lesions. During a mean follow-up of 5.8 years, none showed a recurrence or developed lymphoma. Familiarity with the range of histologic features characteristic of the RT is critical in avoiding misinterpretation as lymphoma [49].

Flow cytometric analysis of CD23 expression in CD5-positive B cells is a widely applied method in the differential diagnosis of CLL and MCL. According to the most accepted criteria, the leukemic cell population is CD19/CD5/CD23 triple positive in CLL but CD23-negative in MCL. Recently, several groups have reported CD23-positive MCL cases; however, these studies mostly analyzed only CD23 positivity but not intensity. Barna et al. compared 26 cases of MCL and 84 cases of CLL using flow cytometric analysis and found that high values of CD23 positivity (>92.5%) and/or high fluorescence intensity (>44.5 mean fluorescence intensity [MFI]) of CD23 are related to CLL, whereas low CD23 positivity (<30%) is related to MCL. However, cases with intermediate CD23 positivity (between 30% and 92.5%) and lower intensity (<44.5 MFI) can either belong to CLL or MCL. In these cases, additional tests such as FISH analysis of the translocation t(11;14) or immunohistochemical detection of cyclin D1 overexpression are required to differentiate CLL from MCL [50].

The morphologic findings of different types of marginal zone B cell lymphoma (MZL) involving the bone marrow (BM), including 18 splenic (SMZL), six extranodal (MALT lymphoma), and six nodal cases were compared by Inamdar et al. The median percentage of BM involvement was 15%, and multiple overlapping patterns of infiltration were observed in all MZL types. The most frequent patterns were nodular (87%) and interstitial (63%). A focal sinusoidal pattern of involvement was found in one third of SMZLs and rarely in MALT lymphoma. Germinal centers (GCs) were uncommon in routinely stained BM biopsy sections and were observed only in SMZL. However, antibodies specific for CD21 and CD23 highlighted follicular dendritic cells in most MZLs of all types. MZLs cannot be distinguished from each other by examining BM sections alone. However, a sinusoidal pattern or presence of GCs is suggestive of SMZL [51].

A rare but relevant pitfall was described by Holanda et al., namely, a primary gastric T cell lymphoma morphologically similar to the gastric marginal zone B cell lymphoma of MALT. Careful interpretation of the immunohistochemistry with additional clonality testing will solve such cases [52].

Normal precursor B cells or hematogones share morphologic and immunophenotypic similarities with lymphoblasts of precursor B lymphoblastic leukemia. The numbers are often increased and difficult to distinguish in many patients following chemotherapy for precursor B lymphoblastic leukemia. The purpose of the study of Hurford et al. was to establish a method for differentiating hematogones from lymphoblasts by evaluating the immunofluorescence pattern of nuclear terminal deoxynucleotidyl transferase (TdT) staining in 29 cases of TdT+ acute leukemia and 20 cases with increased numbers of hematogones. All 29 cases of TdT+ acute leukemia demonstrated a finely granular pattern of TdT immunofluorescence that was uniformly distributed in the nucleus, whereas all 20 cases with increased hematogones demonstrated a coarsely granular or speckled pattern of TdT immunofluorescence, which often intensely aligns the nuclear membrane. The nuclear pattern of immunofluorescence using antibodies to TdT is an effective method for distinguishing hematogones from leukemic blasts [53].

Large CD30-positive cells are the hallmark of cutaneous CD30-positive lymphoproliferative disease, but are not specific for that diagnosis. Werner et al. studied 28 cases of benign cutaneous infiltrates, mainly drug- or virus-induced lesions, and found clusters of such cells with Golgi staining of the CD30 in most of these. It is, therefore, crucial that clinical and pathological data are evaluated with care before the diagnosis of lymphomatoid papulosis or large cell anaplastic lymphoma of the skin, the two ends of the spectrum of cutaneous CD30-positive lymphoproliferative disease, is made [54].

Treatment of patients before a biopsy is taken is sometimes necessary, but may influence to morphology making a diagnosis difficult. Porter et al. evaluated their experience in 109 patients with primary central nervous system lymphoma (PCNSL). Sixty-eight (63.6%) patients received corticosteroids before diagnosis. Thirteen patients (of 109; 12%) had undergone repeat brain biopsy to confirm PCNSL. These included eight (of 68) patients who had received corticosteroids (12%), and five (of 39) who had not (13%) (p=1.0). Therefore, the majority of PCNSL patients who received corticosteroids before diagnosis could be reliably diagnosed on the first biopsy and did not require second biopsy for diagnosis [55].

Diagnosing two different diseases in one patient is always difficult. The coexistence of T cell large granular lymphocyte leukemia (T-LGL) with B cell abnormalities has been described previously. Viny et al. performed a systematic analysis to determine the frequency of this coassociation on 63 T-LGL and identified coexisting B cell dyscrasias in 17 T-LGL patients (27% of total), of whom 12 had monoclonal gammopathy of unknown significance (MGUS) (19%) and five had CLL (8%). The presence of both MGUS and CLL was found in two patients (3%) and follicular lymphoma was identified with MGUS in another T-LGL patient (2%). Additionally, polyclonal hypergammaglobulinemia or hypogammaglobulinemia was found in

10 additional LGL leukemia patients bringing the total frequency of B cell abnormalities in T-LGL leukemia to 43%. The coassociation of B cell pathology with T-LGL suggests that either a common antigen drives clonal B and T cells or that humoral malignancy could serve as the stimulus for lymphocyte expansion representing an overactive antitumor surveillance [56].

Aberrant expression of cytokeratins (CK) is known to occasionally occur in malignant lymphomas. The monoclonal mouse antihuman CK cocktail CK22 recognizes keratin polypeptides with a wide range of molecular weights and can be applied in diagnostic panels for tumors of unknown origin. Using tissue microarray technology, 1,059 lymphoma and acute leukemia cases, covering the most common disease entities, were tested for aberrant CK expression using CK22. In total, 866 of the arrayed cases were evaluable (80%) and 13 positive cases (1.5%) were found: one out of 230 HL (0.4%), one plasma cell myeloma, two out of 326 DLBCLs (0.6%), five out of 18 MCL (26%), three out of 70 SLL/CLL (4%), one out of 27 peripheral T cell lymphomas, and not otherwise specified (4%). Immunostaining was finely granular in most cases, and the total amount of positively staining cells exceeded 10% only in the cases of HL and plasmocytoma. All CK22-positive cases, except for one MCL, expressed the specific simple epithelial CK8 but not the basal/stratified epithelial CK5/6. Aberrant CK expression can be encountered in a small subset of otherwise characteristic B and T cell lymphomas, but not in acute leukemias [57].

Prognostic factors in lymphoma

Adult T cell acute lymphoblastic leukemia (T-ALL) continues to represent an unfavorable disease. Molecularly based treatment stratifications could help improve outcome. The prognostic impact of HOX11 and HOX11L2 expression has been an area of controversy. In 286 adult T-ALL patients enrolled into the German Multicenter ALL therapy protocols, high HOX11 expression and HOX11L2 expression were predominantly seen in thymic T-ALL. In a multivariate analysis, HOX11L2 expression proved to be an independent adverse risk factor for relapse-free and overall survival. HOX11 expression was found to have a favorable impact on relapse-free but not overall survival. Thus, patients with aberrant HOX11L2 expression should be considered early as candidates for intensified treatment regimes [58].

In 67 patients with extranodal marginal zone lymphoma of the stomach, Yamamoto et al. analyzed factors that predict tumor regression upon *H. pylori* eradication. In 28 (42%) of the cases, the tumor cells expressed CXCR3, a chemokine receptor involved in B cell homing. This expression was related to unresponsiveness for eradication

of *H. pylori*, and although CXCR3 expression is correlated to t(11;18), a known marker for unresponsiveness, it remained its prognostic impact in a multivariate model [59].

MCL is a very popular lymphoma for research. Hartmann et al. studied expression levels of 33 genes by q-RTPCR in 73 patients with MCL and were able to create a profile that gave superior prognostic information compared to Ki67 staining, a known strong prognosticator. Five genes from this profile were studied in paraffin samples of 54 cases and the findings were comparable [60]. It will, however, be difficult to translate these results in clinical practice as is exemplified by the situation in DLBCL where profiling about a decade ago has resulted in the recognition of two subgroups, activated and germinal center types, which are prognostically very different. Many studies have used immunohistochemistry to recognize these groups, but only to limited success. Uccella et al. used the most common approach with CD10, Bcl-2, BCL6, and MUM1 antibodies in 71 patients and could not find prognostic relevance, although BCL6 expression as single marker was [61]. Likewise, Katzenberger et al. analyzed cytogenetic alterations in 223 cases of MCL by FISH and/or classical cytogenetics and correlated the results with Ki67 expression and survival. Complex aberrations are associated with high proliferation and poor prognosis. A comprehensive analysis of biological features including genetic alterations in MCL by hierarchical clustering resulted in the delineation of four tumor subgroups differing with respect to their genetic constitution and suggesting different transformation or progression pathways. Moreover, in one of the groups identified, a more indolent clinical behavior was associated with few secondary aberrations and fewer known high-risk chromosomal aberrations [62]. More straightforward is the study by Zanetto et al. who analyzed 17 samples from 14 patients with MCL with aberrant expression of CD10. Five of the cases had blastoid morphology, also five were CD5 negative and BCL6 was expressed by another five, showing that the diagnosis of MCL requires knowledge of the full spectrum of the disease [63].

In the large series of studies dealing with p53, Young et al. adds data that show that the 102 cases with p53 mutation out of 477 patients with DLBCL have a significantly worse prognosis. They were able to show that the location of the mutation affects the prognostic impact. Multivariate analysis confirmed that the International Prognostic Index and mutations in the DNA-binding domains of p53 were independent predictors. TP53 mutations also stratified patients with germinal center B cell-like DLBCL, but not nongerminal center B cell-like DLBCL, into molecularly distinct subsets with different survivals [64].

It is now broadly accepted that DLBCL can be subdivided into the prognostically significant groups germinal center B cell-like (GCB) and activated B cell-like

(ABC) with a less-characterized third groups. Liu at al. immunostained 163 de novo DLBCLs from Chinese patients with CD20, CD10, BCL6, MUM1, CD138, Bcl-2, Ki-67, cyclin D3, geminin, and P27(Kip1). One hundred forty-nine of 163 DLBCLs could then be classified into GCB group (pattern A), activated GCB group (pattern B), and activated non-GCB group (pattern C) according to the expression of CD10, BCL6, MUM1, and CD138. Of the 149 cases, 40 (26%) showed pattern A expression and were grouped as GCB group, lower than the reported frequency of the studies involving mostly Western population. Compared with cases with pattern A, those with patterns B (activated GCB group) and C (activated non-GCB group) more often presented with more aggressive tumors and a shorter survival time. These results indicate that most of DLBCLs from Chinese patients can be classified into prognostically different groups based on the antigenic expression models using a panel of GCB- and ABC-associated markers. Polymerase chain reaction (PCR) analysis of t(14;18) showed that 11 of 64 cases were t(14;18)-positive, and most (10 of 11) of it occurred in the group with pattern A. The translocation was significantly associated with the expression of the Bcl-2 protein. The group with pattern B demonstrated more frequent expression of Ki-67, cyclin D3, and geminin and showed higher proliferative activity than the group with pattern A. These findings suggest that high proliferative activity of tumors with pattern B may be associated with aggressive tumor behavior and poor clinical outcome in patients with DLBCL [65].

As mentioned above, gene expression profiling on frozen tissues has identified genes predicting outcome in patients with DLBCL. Rimsza et al. were able to show that a similar approach is feasible in paraffin tissue in almost all 209 analyzed cases. A practical approach was chosen by Rimsza et al. who analyzed the methylation status of a large series of cases from Tunisia and showed that, in 46 cases of DLBCL, hypermethylated P16, VHL, DAPK, and SHP1 indicate a biologically aggressive phenotype and worse prognosis. Hypermethylation of DAPK was found to be an independent prognostic factor that may be used in conjunction with the conventional prognostic factors such as the IPI and the germinal center status [66].

Bcl-2 associated athanogene-1 (BAG-1) is an antiapoptotic protein as well as a regulator of cell growth. Ataollahi studied 30 DLBCL cases and found nuclear expression in all of the cases and cytoplasmic staining in 83%, whereas nonneoplastic lymph nodes have little staining. This marker revealed no prognostic impact [67].

Since it is relatively easy to perform miRNA profiling in paraffin material, a large number of studies using this approach can be expected in the coming months. Roehle et al. showed that a miRNA profile obtained from 58 cases of DLBCL, 47 follicular lymphomas, and seven nonneoplastic

lymph nodes can be used to create a highly reliable diagnostic tree [68]. It remains to be seen when this approach will replace hematopathologists who were still being used as the gold standard in this study.

Hepatitis B virus (HBV) infection in DLBCL patients is a common complication in China. However, the clinical relevance of HBV infection with respect to DLBCL disease stages and patient survival remains unclear. Compared with the HBsAg-negative group, the HBsAg-positive DLBCL group displayed a younger median onset age (46 vs. 51 years), more advanced stage at grade III/IV (58% vs. 42%), and more frequent hepatic dysfunction before (21% vs. 5.5%) and during (49.4% vs. 16.6%) chemotherapy. Female DLBCL patients exhibited a higher frequency of HBsAg positivity. However, in both groups, the median overall survival (OS) duration (55.8 vs. 66.8 months) and response rates (91% vs. 90.4%) were similar. In the HBsAg-positive DLBCL group, the poor prognostic factors were advanced stage and hepatic dysfunction during chemotherapy. The OS of HBsAg-positive patients with hepatic dysfunction during chemotherapy was significantly shorter than those without liver dysfunction and the OS rates at 3 years were 48% and 72%, respectively. The use of rituximab did not increase the rates of liver dysfunction in HBsAg-positive DLBCL patients. This study suggests that prophylactic treatment of HBV may be of great importance in the cases of HBsAg-positive patients [69].

As was described in the previous review [4] extranodal nasal type natural killer cell lymphoma (ENKL) is common in East Asia. Xiang-Lan analyzed the expression of Skp2 and p27 proteins in 48 cases of ENKL and found that the Skp2/p27 expression profile was an independent prognostic factor for overall survival in this highly malignant disease [70].

Staging

Many pathologists now use routine immunostaining on bone marrow biopsies taken for staging lymphoma patients, and in my experience, enhances recognition of not only limited but also extensive but dispersed involvement by lymphoma. Talaulikar et al. addressed this topic by studying 156 patients with DLBCL using CD20 and CD79a as lymphoma markers. An additional 11% positive cases were detected and these cases had a survival rate comparable to that of H&E detected positive cases, supporting the routine use of immunohistochemistry in evaluating bone marrow biopsies for staging [71].

Bone marrow biopsies (BMB) are the conventional staging method for assessing marrow involvement by lymphoma. Morphological criteria provide basic data determining their dignity, but concerning microfocal infil-

trates, these criteria are rather inaccurate. By examination of immunoglobulin H (IgH) receptor rearrangement and comparison of medullar and nodular lymphoma sites, diagnostic reliability was improved. Employing nonnested IgH rearrangement analysis with FR3A and JH consensus primers, B cell clonality was assessed on glutardialdehyde fixed, decalcified BMB with and without lymphoma infiltration and on the corresponding lymph node specimens. Comparison of lymph node tissues and BMB revealed an identical pattern in 50% of the probes. In 25% of the cases, a single clonal peak derived from the lymph node tissues was also observed in the BMB but was surrounded by additional peaks. IgH FR3 PCR analysis is a suitable tool to examine small lymphoid infiltrates in BMB, and direct comparison with corresponding nodal lymphoma can further facilitate estimation of their dignity [72].

Limited data have shown that immunostaining of bone marrow biopsies improves staging in anaplastic large cell lymphoma by highlighting limited involvement. This is now common practice in such patients and required for inclusion in multicenter trials. Based on a series of 41 patients, Weinberg et al. conclude that immunostaining with CD30, EMA, ALK1, and granzyme has only minor additional value above H&E staining [73].

The increasing application of multicolor flow cytometry assays for staging and follow-up in MCL necessitates that the specificity and sensitivity of this technique are evaluated. Bottacher et al. used standardized four-color flow cytometry assay on 281 prospectively collected peripheral blood and bone marrow samples from 98 patients with MCL participating in a multicenter clinical trial and compared the results to those obtained with conventional clinical staging and consensus primer IGH PCR. Both techniques detected about 10% more stage 4 patients than conventional staging. The sensitivity of four-color flow cytometry is comparable to that of IGH PCR at initial staging but is less sensitive at follow-up after immunochemotherapy. Both techniques are highly valuable methods for accurate initial staging [74].

Ancillary techniques

Using cytologic imprints from spinal lesions of 101 patients, Rezanko et al. were able to speed up the diagnostic process so that early treatment could be installed preventing further damage to the spinal cord. Especially the diagnosis of plasmacytoma, lymphoma, or infectious disease was made with great accuracy [75].

Flow cytometry is increasingly used for the classification of lymphomas. Kost et al. describe the immunophenotype of splenic marginal zone lymphoma (SMZL), nodal MZL (NMZL), and extranodal MZL (MALT) and compared the results to those of follicular lymphoma (FL). All 31 cases of MZL (seven SMZL, six NMZL, 15 MALT, three MZL not otherwise specified) and 31 cases of FL expressed CD19, CD20, and CD45. Thirty-two percent of MZL and 77% of FL expressed CD38. Expression of CD11c was seen in 57% of SMZL and 8% of other MZL ($p<0.01$). Statistically significant differences in antigen expression between MZL and FL were seen for CD10, CD11c, and CD38. MZL expresses typical pan-B cell antigens. Expression of CD11c is highly associated with SMZL. Although no specific phenotype was found for MZL, levels of CD19 expression in conjunction with CD11c and CD38 expression can distinguish MZL from CD10 negative FL [76].

In a more general approach toward the value of flow cytometry in diagnosing and classifying malignant lymphoma, Nguyen developed a Web-enabled relational database integrated with decision-making tools for teaching flow cytometric diagnosis of hematologic neoplasms. This database has a knowledge base containing patterns of 44 markers for 37 hematologic neoplasms. Immunophenotyping data published in the scientific literature were incorporated into a mathematical algorithm that is integrated to the database for differential diagnostic purposes. The algorithm takes into account the incidence of positive and negative expression of each marker for each disorder. The algorithm developed in this database shows significant improvement in diagnostic accuracy over a previous database prototype. This Web-based database is proposed to be a useful public resource for teaching pathology trainees flow cytometric diagnosis [77].

Clonality testing is common in lymphoma diagnosis, but pitfalls have to be known [78]. Since cHL is a clonal B cell neoplasm, Chute et al. used the BIOMED-2 group set of IGH multiplex on 42 cases of cH. The densities of Reed–Sternberg cells/10 high-power field and CD30+ cells/10 high-power field were classified as low, intermediate, or high. The quantities of background CD20+ B cells were classified as low or high. DNA from formalin-fixed, paraffin-embedded sections was used. Overall, 10/42 (24%) of the cHL samples were monoclonal and 7/42 (17%) were borderline monoclonal. Higher densities of CD30+ cells and lower background B cells were statistically correlated with clonality. The BIOMED-2 primers demonstrate IGH gene clonality in 24% to 40% of cHLs without microdissection. In a subset of the cHL, the IGH gene rearrangement analysis might be useful for diagnosis, but can lead to confusion between cHLs and NHLs if used as a discriminative criterion [79].

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