New developments in the pathology of malignant lymphoma. A review of the literature published from August 2010–October 2010

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Introduction

At the Uppsala European Association of Haematopathology/Society of Haematopathology many haematopathologists discussed new findings and difficult cases. At a time where there seems to be limitless access to infinite amounts of information, it seems an anachronistic event. Yet, to be in the city of Lineus, the father of classification and Celsius, the father of measuring along a defined scale and many other famous scientists, to meet in person, to see the faces behind the names on books and articles gives a very different and appropriate feel. Many articles that were reviewed in this series of literature overviews were absorbed as common knowledge, others were forgotten or dismissed. Some were even presented. Also, this issue has lots of new information, some of it surprising, some maybe far from becoming important, some that we can use tomorrow. Still, it remains a stepwise process by which we start to understand lymphomas, how they develop, can be diagnosed and treated.

Biology of lymphoma

B-cell lymphomas

Like any form of cancer, lymphomas develop through a series of genetic changes. Several studies provide further insight in this process. Radiation induces malignancies including malignant lymphomas. It is thought that DNA damage is a direct consequence of radiation and that one of the protecting mechanisms is apoptosis of damaged cells. In contrast, two studies [1, 2] found that mice that lack Puma, resulting in absence of p53-induced apoptosis, have increased lymphoma formation and suggest that this is due to a lower stress-induced proliferative response.

Activation of Rho-GTPases induces B-cell lymphoma, and glutaminase is involved in this. Wang et al. [3] describe a small molecule that inhibits this pathway in neoplastic cells, but not in normal cells.

De-ubiquitinating enzymes (DUBs) can reverse the modifications catalyzed by ubiquitin ligases and, as such, are believed to be important regulators of a variety of cellular processes. Several members of this protein family have been associated with human cancers; however, there is little evidence for a direct link between deregulated de-ubiquitination and neoplastic transformation. Ubiquitin C-terminal hydrolase (UCH)-L1 is a DUB of unknown function that is overexpressed in several human cancers. Hussain et al. [4] generated mice that overexpress UCH-L1 under the control of a ubiquitous promoter resulting in lymphomas and lung tumors. Furthermore, UCH-L1 overexpression strongly accelerated lymphomagenesis in Emu-Myc transgenic mice.

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Other studies investigate which molecular pathways are altered in lymphomas, and several try to influence these pathways by specific targeted molecules. Gastric marginal zone lymphoma (MZL) is partly driven by *Helicobacter pylori*. Lin et al. [5] use B-lymphocytes for introducing the CagA gene. Their result is similar to findings in gastric epithelial cells, which have translational deregulation due to CagA. It is, however, debatable whether the same mechanism is relevant in gastric MZL and in gastric adenocarcinoma: the lymphoma cells are driven by *H. pylori*-specific T-cells and eradication is effective only in the lymphoma situation.

Aberrant signal transducer and activator of transcription 3 (STAT3) signaling participates in the development and progress of human cancers, including diffuse large B-cell lymphoma (DLBCL). Lyu et al. [6] found that the activated B-cell-like (a)/DLBCL lines express higher levels of interleukin-6 receptor (IL-6R) and STAT3 than the germinal center B-cell-like (GCB)/DLBCL lines. Treatment of DLBCL cells with the fusion toxin rGel/BlyS resulted in downregulation of IL-6R and inhibited STAT3 phosphorylation, STAT3-DNA binding activity, and IL-6-inducible STAT3 reporter gene activity. These results indicate that rGel/BlyS is a candidate for the treatment of DLBCL and especially aDLBL.

Daniels et al. [7] measured the expression of all 39 HOX genes in a panel of six malignant B-cell lines, including multiple myeloma cells and found different levels of expression of HOX family members suggesting that they also have a role in malignant B-cell survival. Disrupting HOX function using the peptide HXR9 induced significant cytotoxicity in the entire panel of cell lines, which was enhanced by combining it with ch128.1Av, an antibody–avidin fusion protein specific for the human transferrin receptor 1 (CD71). Iron starvation induced by the fusion protein contributes to the enhanced effect and involves, at least in part, the induction of a caspase-independent pathway.

Leptin has been shown to be overexpressed in various lymphomas. Arrate et al. [8] studied normal B-cells and B-cell lymphomas and describe that leptin inhibits apoptosis and facilitates cell cycle, by inducing expression of bcl2 and cyclinD1. Two pathways are involved, direct activation of their promoters and suppression of microRNAs (miRNAs) that target their putative 3' untranslated regions.

Other approaches are looking into specific genes and/or tumor types. Two papers address Myc-induced lymphomas and focus on microRNAs (miRNA). In general, tumors have decreased levels of miRNA. Dicer controls maturation of miRNA and has a tumor suppressor function in non-haematopoietic cells. MYC suppresses multiple miRNAs in lymphoma cells. Lam et al. [9] found, however, that Dicer and MYC have no synergistic effect on lymphoma development, but rather the opposite. Deletion of Dicer in established B-cell lymphomas resulted in apoptosis, revealing that Dicer is required for B-cell lymphoma survival.

Thus, Dicer does not function as a haploinsufficient tumor suppressor in B cells and is required for B-cell lymphoma development and survival.

Onnis et al. [10] investigated Burkitt lymphomas without a c-Myc rearrangement and show that especially hsa-miR-9-1 gene is heavily methylated in those cases and that this gene indeed influences MYC expression.

To get a better insight in how the Epstein–Barr virus (EBV) results in lymphomas, peripheral blood cells of EBV-positive individuals were put into severe combined immunodeficiency (SCID) mice [11]. The mice developed lymphomas that were mostly EBV-positive, had high p53 protein but no mutations. One wonders whether such findings add to results obtained by studying human tumors. Luan et al. [12] studied indeed human samples (and cell lines thereof) of another virus-induced B-cell malignancy, primary effusion lymphoma (PEL). Using CGH arrays on 28 PELs and ten PEL cell lines they found frequent gains of 1q21–41 (47%), 4q28.3–35 (29%), 7q (58%), 8q (63%), 11 (32%), 12 (61%), 17q (29%), 19p (34%), and 20q (34%), and losses of 4q (32%), 11q25 (29%), and 14q32 (63%). Next they identified selectin-P ligand (SELPLG) and coronin-1 C (CORO1C) as the targets of amplification at 12q24.11. Interphase FISH and quantitative polymerase chain reaction (PCR) showed SELPLG/CORO1C amplification (more than four extra copies) and low levels of copy number gain (1–4 extra copies) in 23% of PELs, respectively. Immunohistochemistry revealed strong expression of both SELPLG and coronin-1 C in the majority of PELs, irrespective of their gene dosage. SELPLG is critical for cell migration and chemotaxis, while CORO1C regulates actin-dependent processes, thus important for cell motility. The authors conclude that overexpression in PEL is expected to play an important role in its pathogenesis.

T-cell lymphoma

Fewer studies are available for T-cell lymphoma development. Relatively many studies are done in anaplastic large-cell lymphoma (ALCL), especially in those that carry the t(2;5) resulting in the nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) fusion gene. Lagarrigue et al. [13] show that NPM-ALK expression results in high levels of metalloproteinase-9 (MMP-9) in cell lines and in all ALK-positive ALCL, but only in about a third of the ALK-negative ALCL. Blocking of the MMP-9 resulted in reduce invasiveness of the cells.

Recently a new translocation was found in T-cell lymphomas, resulting in the ITK-SYK fusion oncogene. About 17% of peripheral T-cell lymphomas not otherwise specified (PTCL-NOS) carry this translocation but almost 90% of the PTCL-NOS have overexpression of SYK. Dierks et al. [14] developed a mouse model I which ITK-SYK overexpression

in bone marrow cells result in a CD4+/-, CB8- T-cell lymphoproliferative disease infiltrating in bone marrow, spleen, skin and lymph nodes. The tumor cells were apoptosis resistant and produce high levels of interferon gamma and became clonal upon serial transplantation. The disease could be blocked by SYK inhibitors.

Epidemiology of lymphoma

For epidemiological studies it is important to use internationally the same language. The WHO 2008 classification is an important reference for all pathologists, but the diagnosis needs to be transformed to meaningful codes for optimal standardization. The 2007 InterLymph system is now updated and can indeed serve as such [15].

Hodgkin lymphoma (HL) is a relative common disease when it presents in lymph nodes or mediastinum. When it presents at unusual sites questions arise whether treatment should be altered. Therefore it is relevant that large institutions present their expertise. From the MD Anderson institute an overview of HL presenting in the Head and Neck region has appeared [16], although one may discuss whether the tonsil and adenoid area is really not a similar site as a nodal site. Over a 40-year period they collected 39 cases, five of which were lymphocyte predominant. Their patients presented more often with localized disease compared with patients who have more usual sites of presentation. However, pathology was not different, and corrected for stage treatment response and survival neither. Therefore there is no need to treat these patients differently.

Several viruses have been implicated in the pathogenesis of malignant lymphoma. Kang et al. [17] focus on hepatitis B and hepatitis C and performed a large case control study in Korea. They compared the incidence of both viruses in 3,932 cases of haematological malignancy with 15,562 controls. HBV was present in 636 control patients (4.1%), 333 lymphoma patients (12.4%), and 75 leukemia patients (6.0%). HCV infection was present in 173 control patients (1.1%), 76 lymphoma patients (2.8%), and 18 leukemia patients (1.4%). Co-infection of HBV and HCV was very rare. HBV infection was associated with increased risks for most subtypes of B and T/natural killer (NK)-cell lymphomas, Hodgkin's lymphoma, and acute myeloid leukemia. HCV infection was associated with increased risks for diffuse large B-cell lymphoma, extranodal marginal zone B-cell lymphoma, peripheral T-cell lymphoma, and acute lymphoid leukemia B-cell early pre-B type. HBV seems to have a more important role than HCV in the pathogenesis of specific haematologic malignancies in Korea. Yoon et al. [18] put these data in perspective by describing the epidemiology of lymphomas in Korea, based on 5,318 cases: 66.9% had mature B-cell neoplasms, 12.5% had mature

T/NK-cell neoplasms, 16.4% had precursor lymphoblastic leukemia/lymphoma (ALL/LBL), and 4.1% had Hodgkin's lymphoma. The most common subtypes were DLBCL (30.5%), plasma cell myeloma (14.0%), extranodal MZL of mucosa-associated lymphoid tissue type (MALT lymphoma; 12.4%), B-cell ALL/LBL (11.3%), Hodgkin's lymphoma (4.1%), peripheral T-cell lymphoma unspecified (4.0%), T-cell ALL/LBL (3.9%), and extranodal NK/T-cell lymphoma of nasal type (3.9%). Most subtypes showed male predominance, with an average M/F ratio of 1.3. Most mature lymphoid neoplasms were diseases of adults (mean age 53.5 years); whereas ALL/LBLs were of young individuals (mean age 20.3 years). Overall, therefore, the differences with western countries are not very large.

H. pylori is well known to be associated with gastric MZL, and other bacteria have been described in MZL from other sites. Decaudin et al. [19] perform a surprising analysis with surprising results. They look for *H. pylori* infection in the stomach of patients with ocular MZL. Indeed, they find a higher incidence, 45%, compared to controls, 12%. It remains a speculation how gastritis without lymphoma in the stomach induces ocular lymphoma.

Chen et al. [20] describe 70 cases of primary lymphomas of the upper aerodigestive tract from Taiwan. The male to female ratio was 2:1, and the median age was 61.5 years (range 8–87); 73% of cases occurred in Waldeyer's ring or the oral cavity. Phenotypically, there were 45 (64%) B-cell and 25 (36%) T-cell or extranodal NK/T-cell lymphoma (ENKL) including 42 (60%) diffuse large B-cell lymphomas (DLBCLs), 22 (31%) ENKLs, three unspecified peripheral T-cell lymphomas, two follicular lymphomas (FLs) and one Burkitt lymphoma. EBV-encoded small RNA in situ hybridization (EBER-ISH) was positive in three (7%) of 42 DLBCLs and all 22 ENKLs. Most patients received chemotherapy with or without radiotherapy. The 5-year overall survival (OS) for all patients was 56.3% with B- and T- or NK/T-cell lymphomas at 66.0% and 40.6%, respectively. Univariate analysis revealed that sinonasal presentation, T- or NK/T-cell phenotype, raised lactate dehydrogenase (LDH) activity, and Ann Arbor stage III/IV diseases were associated with prognostically significant higher hazard ratio (HR) of lymphoma-related death. However, only raised LDH remained significant on multivariate analysis. For DLBCLs, only raised LDH was prognostically significant on either univariate or multivariate analysis.

Lymphomas from the gall bladder and extrahepatic bile duct were collected by Mani et al. [21]. They present 14 primary cases. The most common primary lymphoma types were DLBCL, extranodal MZL, B-lymphoblastic lymphoma, and FL. Two cases had features of lymphomatous polyposis. Rare lymphoma subtypes included the extracavitary variant of primary effusion lymphoma and plasmablastic lymphoma. They conclude that primary lymphomas of the

gall bladder and extrahepatic bile ducts show a broad spectrum of disease types, but in many respects mirror the spectrum of primary lymphomas of the gastrointestinal tract.

Two studies deal with breast lymphomas. Yhim et al. [22] analyzed the data from 68 cases from Korea, Avenia et al. [23] 23 from Italy. The results from Yhim et al. indicate that these lymphomas have a better prognosis, especially in case the lymphoma is confined to one breast. They do not use histological classification, however. The patient series of Avenia is remarkable too: all patients underwent surgery for their disease. In this series, 16 cases were DLBL, seven were ENMZL, and the latter had a much better prognosis. OS at 5 and 10 years was 71.4% and 57.1%, respectively, and all recurrences were systemic. The authors conclude, not surprisingly, that the role of surgery in this disease should be limited to get a definitive diagnosis. It is therefore difficult to understand that they state: the surgical treatment must always be oncologically radical (R0); mastectomy must not be carried out as a rule, but only when tissue-sparing procedures are not feasible. Axillary dissection must always be performed for staging purposes, so avoiding the risk of understaging II or IE, due to the possibility of clinically silent axillary node involvement. This is an approach that most institutions would not advocate.

Celiac disease is uncommon in India. Shet et al. [24] collected intestinal T-cell lymphomas, but found only six cases in 10 years. Four of these were enteropathy-associated T-cell lymphoma (EATL), indicating indeed that this lymphoma type is exceedingly rare in regions where celiac disease is rare.

Defining entities

Hodgkin lymphoma

The diagnosis of nodular lymphocyte predominant HL (NLPHL) remains at times difficult. Churchill et al. [25] analyze the value of programmed death 1 (PD1), a marker of follicular helper T-cells, expression in comparison with that of CD57. The focus in this study is on variants with limited nodularity. PD1 positivity was found in the majority of NLPHL cases with a classic nodular architecture (87%) as compared with 50% for CD57 and was particularly helpful in identifying extranodal large atypical cells. NLPHL with diffuse areas showed a gradual decrease in PD-1 reactivity from nodular to diffuse areas, although a significant proportion (40–50%) of cases retained PD-1 positivity also in diffuse areas. In addition, T-cell and histiocyte-rich large B-cell lymphoma and lymphocyte-rich classic HL displayed PD-1 positivity in a significant subset of cases (33–40%), and thus this marker is of limited value in the more difficult cases.

B-cell lymphomas

How about PD-1 as a marker in B-cell lymphomas then? Muenst et al. [26] addressed this question in 403 cases of B-cell lymphomas of various subtypes. PD-1-positive tumor-infiltrating lymphocytes were numerous in extranodal MZL and FL. Their amount decreased from FL grade 1 to grade 3 and to FL with transformation to DLBCL. An increased amount of PD-1 tumor-infiltrating lymphocytes above the prognostic cutoff score (>2.8%) was a positive prognostic factor of disease-specific survival in FL patients. Five percent of the studied 66 chronic lymphocytic lymphoma (CLL)/small lymphocytic lymphoma (SLL) cases showed unequivocal PD-1 positivity of neoplastic cells. PD-1 therefore is in NK-cell lymphomas also of limited diagnostic value and thus remains only very helpful in identifying angioimmunoblastic T-cell lymphoma (AITL). The prognostic value in FL needs confirmation first.

Low-grade EMZL may contain a various number of centroblasts and also may transform to more aggressive DLBL. Criteria that indicate transformation are scarce. Flossbach et al. [27] suggest, based on a series of 27 cases, classified by morphology in low-grade, combined and large-cell MZL, that bcl6 breaks and protein expression may be a good marker for transformation since it is exclusively found in the latter two groups.

To further characterize the genetic defects in FL, Cheung et al. [28] analyzed 25 cases with a battery of genetic techniques, including classical cytogenetics, BAC and SNP arraying, and found many alterations. This study preludes to the enormous amount of gene alterations we are going to find using next-generation sequencing. It is therefore crucial to start such studies with a well-defined question or hypothesis and use the proper power calculations and biostatistics to come to meaningful results, a huge challenge.

Recognition of FL can be difficult, especially in needle biopsies. Younes et al. [29] investigated two recently described germinal centre B-cell markers, human germinal center-associated lymphoma (HGAL), and LIM-only transcription factor 2 (LMO2), in 127 FL patient biopsies (94 nodal, 33 extranodal) and correlated the findings with histologic pattern, cellular composition, grade and additional immunostains (CD20, CD3, CD21, CD10, BCL2, and BCL6). They conclude that the addition of HGAL and LMO2 to the immunohistological panel is beneficial in the workup of nodal and extranodal B-cell lymphomas and the efficacy of HGAL in detecting the follicular, interfollicular and diffuse components of FL is of particular value in the setting of variant immunoarchitectural patterns see below for LMO2 as marker for gc-DLBL.

Separating mantle cell lymphoma (MCL) from chronic lymphocytic lymphoma/leukemia (CLL) is important, but may be difficult using flow cytometry. Kraus et al. [30]

analyze 90 MCL samples from 44 patients for CD11c expression and compare the results with those in 355 CLL samples from 158 cases. Only in two MCL cases, which were also otherwise unusual, dim CD11c was present, whereas 27% of the CLL cases were positive. Thus, when CD11c is positive, it is a strong argument that one is not dealing with leukemic MCL.

MCL variants are more aggressive, especially the blastoid variant. There is no good interobserver variation for recognizing this subtype. Akyurek et al. [31] investigate whether CKS-1B (CDC28 protein kinase regulatory subunit 1B) can be such marker. In four MCL cell lines, CKS-1B was expressed at variable levels and correlated inversely with p27 expression. In MCL tumors, CKS-1B was positive in 10 (28.6%) of 35 typical versus 14 (87.5%) of 16 blastoid/pleomorphic cases. CKS-1B expression did not correlate with p27 expression, proliferation rate, or prognosis in the entire study group. The mechanism for this expression is unknown and not due to high level amplification. Although the data are statistically significant, this marker is not useful in routine practice.

Durnick et al. [32] studied the relationship between expression of the germinal center marker LMO2 and the presence of IGH-BCL2 fusions, BCL6 translocations, and LMO2 translocations. In addition, they investigated the usefulness of LMO2 expression as a marker of GCB subtype in DLBCL. Immunohistochemical and fluorescence ISH studies were successfully performed on 101 cases of de novo DLBCL that had been incorporated into a tissue microarray. There was a statistically significant association between IGH-BCL2 fusion and LMO2 protein expression but not between BCL6 translocations and LMO2 expression. LMO2 translocations were not identified. The findings suggest LMO2 as a potential marker for the GCB phenotype, but since there were no gene expression data, the real value remains unknown.

Li et al. [33] investigate 17 DLBL of the testis with immunohistochemistry using the Hans algorithm and find 16 of them being aDLBL with high proliferative index.

Gomyo et al. [34] collect seven CD56-positive DLBL. The frequency of this expression profile is 7%, and these cases can have other aberrant expression, one had CD7, another CD8. CD5 was negative in all cases. Bcl6 was positive in six out of seven. All of them had extranodal involvement. It remains to be seen whether CD56 expression is a relevant issue in DLBL.

Dendritic cell neoplasms

There have been two case reports on the presence of *t* (14;18) in dendritic cell sarcoma, one of which was associated with an FL. According to Wang et al. [35], there was no bcl2 break in their four cases.

Dorfman et al. [36] investigated expression of TIM-3 and TIM-4 on normal and neoplastic cells of the monocytic-macrophage lineage. The T-cell immunoglobulin mucin (TIM) proteins are a family of cell surface phosphatidyserine receptors that are important for the recognition and phagocytosis of apoptotic cells. TIM-4 is expressed by macrophages and dendritic cells, and there is moderate to strong staining in juvenile xanthogranuloma and histiocytic sarcoma and lower-level staining in interdigitating dendritic cell sarcoma, Langerhans cell histiocytosis, acute monocytic leukemia and blastic plasmacytoid dendritic cell neoplasm. TIM-3 was expressed by peritoneal macrophages, monocytes and splenic dendritic cells and is also expressed in a range of histiocytic and dendritic cell neoplasms, typically with strong immunohistochemical staining. Cases of diffuse large B-cell lymphoma, ALCL, metastatic malignant melanoma, and metastatic poorly differentiated carcinoma generally exhibited negative to minimal heterogeneous staining for TIM-4 and TIM-3.

New entities/subtypes

Lymphomatoid papulosis (LyP) is a diagnosis made using clinical features combined with histopathology and the lesion belongs to a spectrum of CD30-positive neoplasm of the skin. The cases may resemble histiocytic tumors (type A), mycosis fungoides (type B) or ALCL (type C). Saggini et al. [37] propose a fourth type, D, based on a description of 26 biopsies from nine patients that resembles primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma (CAETL). All cases had at least one cytotoxic marker expressed, and in eight out of nine patients, the tumor cells were CD30-positive. None of them developed systemic disease, which is common in CAETL. Also, in this type, the diagnosis can only be made by combining pathology with clinical features. This is not only important in skin lesions.

Pitfalls in lymphoma diagnosis

Also Takeuchi et al. [38] stress the need for clinicopathologic interaction, but now in gastric lesions. They describe ten cases of lymphomatoid gastropathy as a self-limiting pseudomalignant NK-cell proliferation. These were lesions of about 1 cm, with medium- to large-sized lymphoid cells in the lamina propria and sometimes infiltrating in the epithelium. The cells expressed CD3, but they were CD4- and CD8- negative and partially CD2-positive. CD56 and cytotoxic markers were invariably positive. Most cases were diagnosed as lymphoma, which is correct based on the accepted criteria, although the absence of EBV makes nasal

NK/T cell-type unlike. The authors discuss that these are most often regressing without treatment.

Two tumors in one patient remains a pitfall although each of us has diagnosed a lymphoma in a lymph node removed for staging of a solid cancer. Verwer et al. [39] analyze the occurrence of malignant lymphoma in melanoma patients, a category that is now commonly subject to sentinel node procedures. Of 18,226 patients with melanoma, 55 (0.3%) had lymphoma which is 3.5 times more than expected. Lymphoma was diagnosed subsequent to melanoma in 23 (41.8%) patients, prior to melanoma in 7 (12.7%) patients, and concurrently with melanoma in 25 (45.5%) patients. The lymphoma types were variable with a distribution similar to that in nonmelanoma patients. Chances to find a lymphoma is therefore less than in lymph nodes sampled for prostate cancer or other cancers that arise most often in elderly persons.

Prognostic factors in lymphoma

Vascularization of tumors and targeting it is increasingly seen as important. Also, in malignant lymphomas, some studies have been performed and now Alshenawi [40] adds new data. Expression of vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (b-FGF), CD34 and Ki 67 was assessed in 70 cases of B-cell lymphoma and five cases of reactive lesions. There was an association between Ki67 and VEGF and bFGF staining and also with increased vascularization. The authors discuss how lymphomas may become more aggressive as a function of vascularization.

Liu et al. [41] investigated the expression of E2A (immunoglobulin enhancer-binding factor E12/E47), a transcription factor that induces somatic hypermutation and blocks plasma cell differentiation, in 50 cases of low-stage gastric MZL, and they concluded that its expression is related to *H. pylori* eradication response. It is a pity that they do not give data on the presence of the *t*(11;14), a well-known marker of the same.

Obviously, we like to use new tools and markers, but we should not forget old and tested features. Ott et al. [42] investigated 506 DLBL from a recent clinical trial. They used immunohistochemistry, CD5, CD10, Bcl2, HLA-DR, IRF4/MUM1 and Ki 67, the Hans algorithm created thereof and morphology (in 906 patients) to identify a prognostic marker. In this series, in contrast to others from the Rituximab era, there was no prognostic impact of the distinction of aDLBL and GCDLBL; also, individual immunohistochemical markers had no prognostic value. Immunoblastic morphology indicated poor survival, like what was already shown in the premodern therapy time using the Kiel classification. These data confirm that any new study of prognostic markers in DLBL should include at least morphology as well.

Song et al. [43] took a similar approach using data from 136 patients with DLBL, but only looked into the Hans algorithm and Blimp-1. Blimp-1 was detected in 38 (30.0%) patients and was associated with a significantly shorter OS ($P=0.030$). Using the Hans classification based upon the expression of CD10, Bcl-6 and MUM1, 54 patients had GCB phenotype and 82 had non-GCB phenotype. The 5-year OS rate was 75% in the GCB group and 52% in the non-GCB group ($P=0.020$). The positive rate of Blimp-1 was 22.2% in the GCB group and 31.7% in the non-GCB group ($P=0.329$). The Cox regression multivariate analysis showed that international prognosis index (IPI) and Hans classification had independent prognostic significance, whereas Blimp-1 was not an independent prognostic factor.

A study that focuses on genomic aberrations was performed by Scandurra et al. [44] used array CGH data and related those to clinical data from 124 patients with DLBL. They were able to create five subgroups with different clinical features, but since classical markers were not included, it is difficult to value the results.

The role of apoptosis in lymphoma is well studied and several articles describe prognostic relevance of expression of proteins involved in this process. Hussain et al. [45] showed that XIAP is expressed in about 55% of DLBL and that it indicates poor prognosis. Using cell lines, they were able to show that there was a relation to activated AKT and that inhibition of XIAP resulted in apoptosis of the cells.

Recently, the interest in mitochondria in tumor biology has increased. Wilkinson et al. [46] demonstrate both in vitro and in clinical cases that increased expression of cytochrome c correlates with drug resistance and poor survival in DLBL and MCL.

Ancillary techniques

A variety of techniques is being used or developed for arising at a reliable diagnosis with minimal invasive techniques. For example, the use of fine needle aspiration to obtain a cytology specimen from a difficult site is commonly being used. Rossi et al. [47] describe three patients with pancreatic lesions which were diagnosed as lymphoma using FNA with liquid-based cytology and phenotyping.

Clonality testing is a powerful technique, but prone to mistakes to lack of knowledge of the pitfalls. One pitfall is the presence of T-cell clones in B-cell lymphomas. Ibrahim et al. [48] investigated this issue in 26 B-cell post-transplant lymphoproliferative disorders, 23 human immune deficiency virus-associated B-cell lymphomas and 10 immune-competent DLBCLs for B- and T-cell clonality (PCR and heteroduplex analysis using BIOMED-2 protocol), T-cell

subsets (immunohistochemistry) and EBV association (EBER-ISH). About 50% of B-cell post-transplant lymphoproliferative disorders showed evidence of monoclonal T-cell expansion, and among the T cells present in the tissue samples, CD8-positive cells predominated. There was no association between EBV and presence of T-cell clones. T-cell clones were not identified in lymphomas other than B-cell post-transplant lymphoproliferative disorders. It is important to realize, as the authors stress, that this finding does not indicate a post transplant T-cell lymphoproliferative disorder.

It is not so well known that RNA is well preserved in formalin-fixed, paraffin-embedded (FFPE) tissues. The problem is to get it out and use it. Antica et al. [49] present a simple and fast method for RNA isolation from formalin-fixed paraffin-embedded lymph nodes that can be successfully applied for reverse transcription (RT)-PCR as well as for quantitative (q) RT-PCR analysis. They tested diverse isolation reagents and combined a range of factors in order to get a high-quality RNA for retrospective studies of gene expression in human lymphoma samples. Their modified method of RNA extraction from FFPE provides superior yields and purity based on qPCR data.

Few studies have been performed using proteomics to classify lymphomas. Schwamborn et al. [50] used matrix-assisted laser desorption/ionization (MALDI) imaging and were able to separate HL from reactive lesions with a sensitivity and specificity of 83.92% and 89.37%, respectively.

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