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REVIEW OF THE LITERATURE

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

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Introduction

The pathology of malignant lymphoma remains a rapidly evolving field. Based on the long tradition of research into the understanding of the biology of the tumor cells that leads to better definition for clinicopathologic entities, resulting in new methods used by pathologists to diagnose a lymphoma, also in the first 4 months of 2008 a large series of papers was published. In this overview, the most important of these are described shortly.

Biology of lymphoma

Hodgkin lymphoma

Hodgkin lymphoma (HL) remains an elusive disease. Liu et al. described the involvement of defects in deoxyribonucleic acid repair in six out of eight cell lines and three out seven cases, with deletions and insertions in the RaD3-related (ATR) genes and suggest that these might prevent repair induced by oxidative stress of the inflammatory infiltrate in HL, thereby once again pointing to the inflammation as an important driving force of the disease [1]. Diepstra et al. showed that the tumor cells in about half of the HL cases express human leukocyte antigen G, which inhibits cytotoxicity. This expression was associated with loss of major histocompatibility complex class I so that by these mechanisms, Hodgkin cells protect themselves from killing by the inflammatory response [2].

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Using cell lines from HL and anaplastic large cell lymphoma (ALCL), Hirsch et al. showed that Hodgkin cells are unresponsive for CD30 signaling but that in ALCL cells, CD30 stimulation causes major transcriptional alterations, including activation of caspases and nuclear factor-kappaB-mediated survival, in fact competing processes [3].

Microribonucleic acid (miRNA) expression profiling in classic HL (cHL) revealed that the expression of 157 miRNAs in lymph nodes from 49 cHL patients and ten reactive lymph nodes separated three well-defined groups: nodular sclerosis cHL, mixed cellularity cHL, and reactive lymph nodes. The results were confirmed in a separate series of cases and cell lines. These findings suggest that miRNAs play an important role in the biology of cHL [4].

Using mass spectrometry, Ma et al. detected in cells and supernatants of HL cell lines in total 1,290 proteins, including 368 secreted proteins. The secreted proteins included 37 related to immune response. Several of these proteins were also elevated in the serum of patients with HL and may serve as biomarkers after confirmatory studies [5].

All these findings lead to a better understanding of what is happening in HL importantly, since still about 10–15% of the patients eventually die of this lymphoma type.

B cell lymphomas

It is now widely accepted that diffuse large B cell lymphoma consists of at least two phenotypic subtypes, that is, the germinal center B cell-like and the activated B cell-like groups. It has been shown that the former group responds favorably to chemotherapy and expresses high levels of BCL6. But, the latter group has lower levels of BCL6, constitutively activated nuclear factor-kappaB, and tends to be refractory to chemotherapy. Ding et al. showed that the STAT3 gene is a transcriptional target of BCL6. As

a result, high-level STAT3 expression and activation are preferentially detected in the activated B cell group and BCL6-negative normal germinal center B cells [6].

The unusual-type pyothorax-associated lymphoma is a B cell lymphoma developing in the pleural cavity affected by chronic pyothorax. Takakuwa et al. showed by clonality testing and sequencing of the immunoglobulin heavy chain gene in cases and cell lines that this tumor is derived from crippled postgerminal center cells. Usually, such cells do not survive, but the authors suggest that the Epstein–Barr virus (EBV) that is invariably present in these cases might provide the basis for the cell survival [7]. O'Hara et al. detected in this lymphoma type that in addition, specific miRNA gene amplifications and concordant changes in pre-miRNA and mature miRNA were present [8].

By clonality analysis and sequencing of the variable region of the immunoglobulin gene in cases of thyroid lymphoma proceeded by Hashimoto thyroiditis showed that most cases had already rearranged the immunoglobulin gene of the malignant population in the benign precursor lesion, supporting the notion that these lymphomas indeed develop out of the Hashimoto's thyreoiditis [9].

Montesinos et al. used expression profiling to determine the cell of origin of 21 cases of central nervous diffuse large B cell lymphoma and compared the results with profiles from various subsets of normal B cells. The expression profile of the central nervous lymphomas was similar to that of late germinal center B cells, and some were of the activated B cell-like diffuse large B cell lymphoma from other sites and others from the germinal center B cell-like subtype [10].

T-cell lymphoma

Kchour et al. showed in an extensive study on 20 patients with human T lymphotropic virus (HTLV1)-associated T cell lymphoma/leukemia that, as compared to organs from matched controls, increased vascularization was present in all tested involved organs from adult T cell leukemia/lymphoma (ATLL) patients, suggesting that angiogenesis plays an important role in the development or organ invasion of ATLL and could represent a potentially interesting target for antiangiogenic therapy of ATLL [11].

Epidemiology of lymphoma

It is well known that regional differences exist in the epidemiology of lymphoma types. Using the World Health Organization (WHO) classification on 2,260 lymphomas from Japan, Aoki et al. confirmed that follicular lymphoma is rare (18%) compared to that of the Western series but that T/natural killer (NK) cells are quite common (25%). Like in Western counties, diffuse large B cell lymphoma is the most common type (33%). Even within Japan, there are regional differences with some areas with high numbers of HTLV1-associated lymphoma proliferations [12].

A study of 61 Korean cases of follicular lymphoma showed that this type is not only relatively rare in Eastern countries but also that it is more often grade 3 (57%) and has less often a *t*(14;18), namely 44%. Since the cases of grade 3 had the least translocations, it might be that they correspond to the 3B cases in Western countries [13].

Changing the environment may well lead to changes in frequencies of malignancies. Chang et al. show that in 74 cases of HL diagnosed before 1996, EBV was present in 61% but in 99 more recent cases, 39%; the recent cases were also more often of the nodular sclerosing type compared to earlier. The pattern therefore moves toward a more Western one [14].

Defining entities

B cell lymphomas

Knowledge of the state of differentiation of lymphoma cells is very helpful in classification. Germinal center B cell-expressed transcript-1 gene that codes for a serpin expressed in the germinal center (centerin) was characterized in human tissues, cell lines, and a large series of lymphomas. The results demonstrate that centerin is expressed exclusively by neoplasms hypothetically to be arrested at the germinal center stage of differentiation, including follicular lymphoma, nodular lymphocyte-predominant HL, and a subset of diffuse large B cell lymphoma, T cell/histiocyte-rich B cell lymphoma, and Burkitt lymphoma. Chronic lymphocytic leukemia, splenic marginal zone lymphoma, and mantle cell lymphoma are negative; it would be interesting to know the results in marginal zone lymphomas, the lymphoma type that is presently often misdiagnosed (see below) [15].

A proportion of gastric extranodal marginal zone B cell lymphomas of the mucosa-associated lymphoid tissue (MALT) type are dependent on the presence of *H. pylori* Toll-like receptors that recognize bacterial proteins and autoantigens, which results in inflammatory reactions and influences tumor development and growth. Adam et al. investigated the presence of Toll-like receptors 4, 5, and 9 on a series of lymphomas and found TLL-4 to be expressed only on all 19 extranodal marginal zone lymphomas. It is a pity that no data on t(11;18), associated with unresponsiveness for *H. pylori* eradication, are given [16].

It is quite clear that there is a strong relation between cyclin D1 overexpression and mantle cell lymphoma but also that there are cyclin D1-negative mantle cell lymphoma and cyclin D1-positive malignancies of lymphoid

lineage (hairy cell leukemia, plasmacytoma) that may express cyclin D1. Ehringer et al. investigated 231 diffuse large B cell lymphomas for the expression of cyclin D1 and CD5 and found ten cyclin D1-positive and CD5-negative cases. Two of these cases had t(11;14) by fluorescent in situ hybridization, and two cases had amplification of the cyclin D1 signal. The expression of MUM1 and bcl-6 in nine of ten cases confirms that these are not CD5-blastoid mantle cell lymphoma. These data underscore the importance of combining morphology, immunophenortype, and molecular findings in order to reach a correct classification [17].

Brizova showed in a large series of cases that the messenger RNA level of cyclin D1 in 60 of 61 mantle cell lymphoma far exceeds that of other lymphoma types except for mucosa-associated lymphomas. The latter finding was attributed to the presence of epithelial cells in the tested sample. The study did not contain cases of highly cyclin D1-expressing plasmacytomas. It was not clear from the paper that the quantitative reverse transcriptase polymerase chain reaction (PCR) method was superior to immunostaining for cyclin D1 [18].

Using expression profiling, the existence of cyclin D1-negative mantle cell lymphomas was discovered, but these are very difficult to recognize (Fu et al.). Ek et al. described that the transcription factor Sox11 is specifically expressed in the nucleus of mantle cell lymphoma and not in other lymphomas and benign lymphoid tissue. Although the role of Sox11 presently is not known in lymphocyte ontogeny, it is normally expressed in the developing central nervous system in the embryo and shows sequence homology with Sox4, a transcription factor crucial for B lymphopoiesis. If confirmed, this may be an important marker for detecting mantle cell lymphoma, especially cyclin D1-negative cases [19].

The topic of Burkitt and Burkitt-like lymphoma is very hot, and an important one at the European Association of Hematopathology meeting in Bordeaux. There are also several papers that contribute to our knowledge, even though a solution how to diagnose these cases is not yet available.

Chuang et al. describe the pheno- and genotypic profile of 17 sporadic pediatric and 14 adult Burkitt lymphoma and showed, as expected, large similarities. The prognosis, however, is very different, with good prognosis in the pediatric age group and poor prognosis in adults. The authors suggest that this is partially due to an initial wrong diagnosis but mainly due to insufficient treatment. This point warrants further studies [20].

In 72 patients with lymphomas that had Burkitt or Burkitt-like morphology, Nomura et al. analyzed phenotypic and clinical factors and showed that these patients benefit from aggressive short-term therapy, regardless of the presence of the typical Burkitt phenotype (bcl-2−; CD10+; mib 1 >95%) c-myc translocation [21].

Rodig et al. based on the recently described gene expression profiles differentiating B cell lymphoma and diffuse large B cell lymphoma examined a cohort of 67 cytogenetically defined aggressive lymphomas using immunohistochemical techniques for expression of TCL1, CD38, and CD44 and found distinct expression patterns between MYC+ and MYC− tumors that are better predictors of MYC status than combined staining for CD10 and BCL2 [22].

T cell lymphomas

The debate whether ALCL consist of two groups namely, anaplastic lymphoma kinase (ALK)-positive and ALK-negative, cases may be solved by the results of Salaverria et al., since they found clear differences in the respective genomic profiles of these groups, even though there were few specific gene alterations for each group except for the known translocations involving the ALK locus [23].

T/NK cell lymphomas of nasal type are relatively common in Eastern Asia, and it probably is understandable that a series of such cases that occur primarily in lymph nodes was described from Japan. Takahashi collected a series of EBV- and CR56-positive cases of such and compare them with peripheral EBV-positive cytotoxic peripheral T cell lymphomas NOS and primary nasal T/NK lymphomas with secondary nodal involvement. Whereas the nasal cases did not have clonally rearranged T cell receptors, four of six nodal cases had a clonal T cell receptor gamma rearrangement and all the T cell lymphomas. With respect to clinical features, they were only minor differences between the groups. These results reiterate the difficulties there still are in classifying T cell lymphomas in a meaningful way [24].

EBV-associated T/NK-cell lymphoproliferative disorder (EBV-T/NK LPD) of children and young adults is generally referred to as severe chronic active EBV infection (CAEBV). This disease is rare, associated with high morbidity and mortality, and appears to be more prevalent in East Asian countries. But, because there is no grading or categorization system for CAEBV, pathologists and clinicians often disagree regarding diagnosis and therapy. EBV-T/NK LPD includes polyclonal, oligoclonal, and monoclonal proliferation of cytotoxic T and/or NK cells. Moreover, a unique disease previously described as infantile fulminant EBV-associated T-LPD has been identified and overlaps with EBV-T/NK LPD. Oshima et al. propose a clinicopathologic categorization of EBV-T/NK: (1) category A1, polymorphic LPD without clonal proliferation of EBV-infected cells, (2) category A2, polymorphic LPD with clonality, (3) category A3, monomorphic LPD (T cell or NK cell lymphoma/leukemia) with clonality, and (4) category B, monomorphic LPD (T cell lymphoma) with clonality and fulminant course.

Categories A1, A2, and A3 possibly constitute a continuous spectrum and together are equivalent to CAEBV. Category B is the exact equivalent of infantile fulminant EBV-associated T-LPD [25].

Shimauchi et al. hypothesized that adult T cell leukemia/lymphoma derives from regulatory T cells since they express CD4 and CD25. They show that these cases also express the regulatory T cell marker FoxP3, but functional studies revealed that the cells had no suppressor activity toward CD8 cells. This study is important since in general, phenotype is considered sufficient proof of derivation of a lymphoma from a physiological cell, but the point that phenotype and function go not always together needs to be well taken [26].

Cutaneous lymphomas

The classification of cutaneous lymphomas remains a difficult area. Khamaysi et al. applied the WHO–EORTC classification on their series of cutaneous lymphomas and found 43 new nonmycosis fungoides/Sezary syndrome lymphomas; 29 B cell lymphomas of which 14 were follicle center lymphoma, 10 marginal zone lymphoma, 4 diffuse large-B cell lymphoma, leg type, and 1 diffuse large B cell lymphoma, other;14 T cell lymphomas including five cases of lymphomatoid papulosis, two CD30+ anaplastic large cell lymphomas, one NK/T cell lymphoma, and six peripheral T cell lymphomas, unspecified. Of the six "unspecified" T cell lymphomas, three were CD4+ small/medium-sized pleomorphic T cell lymphoma, which is considered currently a provisional entity under the unspecified T cell category. The remaining three cases could not be classified beyond the unspecified T cell category, of which two cases had an aggressive course. They conclude that the new WHO–EORTC classification is applicable to most nonmycosis fungoides/Sezary syndrome primary cerebral lymphoma cases, especially the B cell lymphomas [27].

From their large collection of cutaneous lymphomas, the EORTC cutaneous lymphoma group described their cases of subcutaneous panniculitis-like T cell lymphoma in a landmark paper. This lymphoma type is quite aggressive and consists of cases of the alpha/beta T cell phenotype (SPTL-AB) and with a gamma–delta T cell phenotype (SPTL-GD). The series of 63 SPTL-ABs and 20 SPTL-GDs were studied at a workshop of the EORTC Cutaneous Lymphoma Group. SPTL-ABs were generally confined to the subcutis, had a CD4−, CD8+, CD56-, betaF1+ phenotype, were uncommonly associated with a hemophagocytic syndrome (HPS; 17%), and had a favorable prognosis (5-year overall survival). SPTL-AB patients without HPS had a significantly better survival than patients with HPS. SPTL-GDs often showed (epi)dermal involvement and/or ulceration, a CD4−, CD8−, CD56+/−, betaF1-T cell phenotype, and poor prognosis, irrespective of the presence of HPS or type of treatment. These results indicate that SPTL-AB and SPTL-GD are distinct entities and justify that the term SPTL should further be used only for SPTL-AB. SPTL-ABs without associated HPS have an excellent prognosis, and multiagent chemotherapy as the first choice of treatment should be questioned [28].

Wasco et al. described the expression of MUM1 in a series of 58 lymphomas presenting in the skin. It appeared that CD30-positive lymphoproliferations, primary or secondary, generally express MUM1. Also, mycosis fungoides with CD30-positive component expresses MUM1, but other cases of mycosis fungoides were not [29].

The morphologic spectrum of 66 biopsies from 47 patients with primary cutaneous anaplastic large T cell lymphoma appeared to be very large. The 'common variant' was the most frequent (40%). Marked reactive infiltrates are commonly present, and 26 cases were classified as 'inflammatory type' (15 cases) or 'lymphohistiocytic' (11 cases). Concerning the predominant cell morphology, large anaplastic cells (33%) were almost as frequent as large pleomorphic (36%) and small- to medium-sized cells (26%). There were two rare cases with the predominance of large cells with a 'signet-ring'-like appearance. Epidermotropism and the presence of eosinophils were found in a proportion of cases in all variants [30].

New entities/subtypes

In this time of tailored therapy and multiple techniques, it seems that each patient has his or her own specific subtype of lymphoma. Prof. Lennert advocated that an entity could be accepted as such if its incidence was at least 5% of the lymphomas. This is probably too restricted as exemplified by the subdivision in cutaneous large cell lymphoma in leg type with poor prognosis and follicle center lymphoma with a very good prognosis as proposed by the group of Willemze. This subdivision was debated fiercely but is now generally accepted. Van Galen of the Willemze group now shows that expression profiling separates these subgroups into the leg type with intense cellular cytotoxic response and the primary cutaneous follicle center lymphoma with constitutive activation of the intrinsic-mediated apoptosis pathway, with concomitant downstream inhibition of this apoptosis pathway [31].

The spleen remains an enigmatic organ and so are the lymphomas with primarily splenic involvement. Traverse et al. described 37 cases selected from a group a patients with circulating villous lymphocytes that have numerous basophilic villous lymphocytes. The patients, predominantly older men, presented with moderate lymphocytosis and

splenomegaly without pancytopenia. The monoclonal B cells expressed IgM+D, IgM+G, IgM or IgG, as well as CD76 and CD11c, frequently CD103, and rarely CD123. Spleen sections were peculiar, with atrophic white pulp and a monomorphic diffuse lymphoma infiltration in a congested red pulp. Bone marrow infiltration was interstitial and intrasinusoidal without extensive fibrosis. Cytogenetic analysis showed a frequent absence of clonal aberrations (68%). Most cases (79%) were IgH-mutated, with an overrepresentation of V(H)3 and V(H)4 gene families. These results, as well as the clinical evolution, show that those lymphoma cases represent a homogeneous group distinct from splenic marginal zone lymphoma and reminiscent of hairy cell leukemia variant, perhaps corresponding to a separate lymphoma entity [32].

In a series of 55 cases of nodular predominant HL, Yang et al. described a subset of cases that occur in older individuals, at atypical sites and with an increased number of large cells. It remains to be seen what the outcome of the cases is [33].

Vega et al. described six lymphoma cases characterized by t(8;13), a translocation well known from the 8p11 myeloproliferative syndrome, a rare hematologic malignancy characterized by myeloid hyperplasia, eosinophilia, and precursor lymphoblastic lymphoma. Histologically, each tumor was composed of two distinct cellular components: small- to medium-sized T cells with scant cytoplasm that resembled lymphoblasts and larger immature-appearing cells with more abundant eosinophilic cytoplasm that resembled myeloblasts, a subset of which expressed myeloid antigens. In all cases, the latter component tended to surround residual lymphoid follicles and/or blood vessels. Numerous eosinophils and prominent high endothelial venules were present in all of the lymph node specimens. Interestingly, cells of both components expressed CD3 on immunohistochemical stains [34].

Pitfalls in lymphoma diagnosis

Clonality does not equal malignancy. Germinal centers consist of one to four clones of B cells, and thus clonality assessment leads to oligo- or even monoclonal results. However, the presence of light-chain-restricted plasma cells is unusual in a germinal center. Nam-Cha et al. described eight such cases, two of which were Castleman's disease. As expected, a clonal peak was found at clonality testing in most of these cases. One patient developed a follicular lymphoma, so that at present, the meaning of the finding remains unclear, but certainly, clonality is not sufficient to diagnose lymphoma [35].

Nodal marginal zone lymphoma is a recognized difficult entity, especially since there is no specific diagnostic marker. Especially challenging are cases with prominent follicular colonization. Naresh described 15 such cases, almost all referral cases and all with a different initial diagnosis. One can only recognize these cases if the immunophenotype of the cells follicle centers is assessed with great care. Benign follicle center cells express CD10 and Bcl-6 but not Bcl-2 and MUM1. In contrast, the colonizing marginal zone lymphoma cells express Bcl-2 and often MUM1 but not Bcl-6 and CD10. Partially colonized follicles showed a 'moth-eaten' appearance on CD10, Bcl-2, Bcl-6, and MUM1 immunohistochemistry [36].

Increasingly, bone marrow biopsies are taken with rituximab treatment to evaluate the treatment response. Regularly, infiltrates are found that upon immunostaining consist of T cells only. Taynoud et al. studied such cases from a clinical trial and were able to show that morphology alone is not reliable in assessing these biopsies when infiltrates are found and that, importantly, when infiltrates consist of only T cells the patients have an improved prognosis compared to patients without infiltrates. The authors suggest that the T cell infiltrate might reflect a beneficial immune response against lymphoma cells [37].

Kojima et al. reported on five cases they refer to as idiopathic plasmacytic lymphadenopathy with polyclonal hyperimmunoglobulinemia (IPL), all detected incidentally on routine chest X-rays, with multiple small nodular lesions in the bilateral lungs. Clinically, autoimmune disease was suspected for all five cases, and the various autoantibodies were investigated. Pathologically, all five lesions were characterized by well-demarcated masses that consisted of abundant reactive germinal centers and a dense lymphoplasmacytic infiltrate in the interfollicular area with a variable degree of interfollicular fibrosis. The immunohistochemical study and PCR demonstrated the polytypic/polyclonal nature of the plasma cells and B cells. It is important to discriminate between pulmonary involvement of IPL and marginal zone B cell lymphoma of the mucosa-associated lymphoid tissue type [38].

Prognostic factors in lymphoma

Understanding lymphoma biology and using this knowledge in clinical practice has for many years brought the diagnosis of lymphomas at the forefront of diagnostic pathology, using new technologies early on. This seems not the case anymore since in contrast to solid tumors, predictive factors are not rapidly replacing prognostic factors in the lymphoma field (Her2/neu, KRAS, CD117), with only CD20 as predicting rituximab response. Many papers still appear that describe prognostic factors, but until now, very few have come into clinical practice. Van Galen et al. described that expression of tumor necrosis factor receptor-associated factor 2 correlates with poor prognosis in a group of 20 activated B cell-like-type diffuse large B cell lymphoma but not in the 13 tested germinal center type

[39]. LMO2 protein expression predicts survival in 263 patients with diffuse large B cell lymphoma treated with anthracycline-based chemotherapy including those 80 that had in addition rituximab [40].

In follicular lymphoma, germinal center-like diffuse large B cell lymphoma, and HL, many FOXP3+ regulatory T cells are associated with improved survival [41].

Martini et al. showed that expression of phosphorylated STAT5 in cHL is associated with better survival compared to negative cases [42].

For follicular lymphoma, a gene expression profile was determined by Lebrun et al. that predicts poor outcome [43]. Diaz-Alderete et al. showed that clinical features are related to the presence of bcl2 and bcl6 translocations [44]. Using samples from 194 follicular lymphoma patients, Cainioni showed that the poor prognostic impact of high numbers of macrophages in patients treated without rituximab disappears when rituximab is given [45].

It is well known that proliferation is an important prognostic factor in mantle cell lymphoma. Using data from a large clinical trail that used rituximab treatment, Ki67 immunostaining remained prognostically relevant: The three groups with different Ki67 index of less than 10%, 10% to less than 30%, and 30% or more showed significantly different overall survival in patients treated with CHOP as well as in patients treated with CHOP in combination with anti-CD20 therapy [46].

D'Haene et al. showed that in 58 primary central nervous system lymphomas, of the commonly observed features necrosis, reactive perivascular T cell infiltrate and endothelial hyperplasia, only the latter (present in 21% of the cases) has a prognostic impact, but only in immune-competent patients. Furthermore, endothelial galectin-3 expression but not galectin-1 expression indicated poor prognosis, in immunocompetent cases only [47].

Patients with refractory celiac disease are prone to develop enteropathy-associated T cell lymphomas. Verbeek et al. show that the aberrant phenotype with a cutoff of 20% as detected by flow cytometry predicts this development better than clonality testing [48].

Staging

The group of Jong studied the staging and clinical data of 106 patients with primary extranodal nongastric marginal zone MALT lymphoma. They confirm that this process frequently presents as stage IV disease (26%) and multifocal disease (32%) and with a site-specific dissemination pattern. They proposed after an extensive staging procedure at presentation to use primary site-directed protocols during follow-up focused on the primary-involved tract/organ system, regional lymph nodes, and pulmonary or gastric relapses [49].

Staging of lymphoma patients remains a cornerstone in clinical management. Bone marrow involvement is often a determining criterion but is not done optimally in many cases. In a series of 113 cases of diffuse large B cell lymphoma from one institution, Talaulikar et al. showed that flow cytometry detected involvement in seven cases that were negative by histology but also that 11 cases were missed. Their results also show that combining the positive cases from conventional staging and flow cytometry has the best predictive value for survival. These data are difficult to interpret, since staging has an influence on treatment [50].

Increasingly fluorodeoxyglucose (FDG) positron emission tomography (PET) is being used in the staging procedure of lymphoma patients. Pelosi et al. addressed the question whether this technique can replace bone marrow biopsies in 194 patients. Although PET and biopsies had similar sensitivity and accuracy, bone marrow involvement was identified by both methods in only 10 out of 49 patients with positive bone marrow involvement by either one of the techniques. There were no significant differences between the HL and the non-HL patients. The authors conclude that FDG-PET has added value to bone marrow biopsies but does not replace them [51].

Ancillary techniques

Less invasive approaches for diagnosing and classifying lymphomas are potentially very important. The proteomic spectra of crude sera from 132 patients with diffuse large B cell lymphoma and 75 controls obtained by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) achieved a sensitivity of 94% and a specificity of 94% for detecting diffuse large B cell lymphoma samples in the test set of 85 samples and achieved a sensitivity of 94% and a specificity of 92% for detecting poor prognosis patients in the test set of 66 samples [52]. Using a similar SELDI-TOF-MS approach on small biopsies/fine-needle aspirations from lymphoma patients, Jansen et al. were able to create protein profiles that separate reactive lymph nodes and low-grade B cell lymphomas from cases of diffuse large B cell lymphoma [53]. Using liquid chromatography/mass spectrometry, Roy et al. analyzed the cerebrospinal fluid (CSF) from patients with central nervous lymphoma and confirmed the results with enzyme-linked immunosorbent assay. Approximately 80 CSF proteins were identified and found to be present at significantly different concentrations, both higher and lower, in training and test studies, which were highly concordant. The findings demonstrate that proteomic analysis of CSF yields individual biomarkers with greater sensitivity in the identification of cancer than does CSF cytology [54].

Lohan et al. went one step further and described the magnetic resonance enterography results of ten patients with small bowel lymphoma and provided features that enable the prediction of the histological type of lymphoma [55].

Lymphoma classification is in some instances based on cytogenetic data, but not many laboratories perform routine cytogenetic analysis on biopsies for lymphoma. Dunphy and Tang analyzed their experience with 261 samples, of which four appeared to be nonhematologic malignancies. In 64 cases, no result was obtained. In 5 of 78 reactive neoplasias, an abnormal cytogenetics was found, and three of these were also clonal and developed follicular lymphoma soon afterwards. Furthermore, in follicular lymphoma, additional cytogenetic features were found to be related to more aggressive grades. Finally, in several cases, the cytogenetic data gave more insight in the process detected [56].

Flow cytometry is commonly used for leukemia classification but is increasingly used for lymphoma diagnosis in conjunction with histological and immunohistochemical evaluation. Karube et al. showed the value of this approach in a series of 490 NK and T cell lymphoproliferations with 15 commonly used markers [57].

Clonality testing in skin lymphomas is considered of little additional value because of the low specificity of the test (Langerak et al.). This was confirmed by Plaza et al. who used clonality testing of the T cell receptor beta gene in a series of 80 cases with a cutaneous infiltrate including reactive dermatoses. They confirmed that almost all cases diagnosed as lymphoma were clonal but that also clonal populations were present in some of the reactive dermatoses [58]. In an important study of Ponti et al., the multiple biopsies approach proved helpful. In many patients, one has more than one biopsy available, and it appeared that in mycosis fungoides, an increase in clonality was observed in connection with both a worsening of the cutaneous disease (79% T1/T2; 100% T3/T4) and an increase in the histopathologic score (HS$<$5, 76%; HS$\geq$5, 94%) [59].

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