PET/CT in Lymphomas

A Case-Based Atlas

John A. Andreou
Paris A. Kosmidis
Athanasios D. Gouliamos
Editors

In collaboration with
Effimia P. Vrakidou
Vassilios K. Prassopoulos
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Ἐς δὲ τὰ ἔσχατα νουσήματα αἱ ἔσχαται θεραπεῖαι ἐς ἀκριβείην, κράτισται

For extreme diseases extreme strictness of treatment is most efficacious
Hippocrates Med. et Corp, Aphorismi 1.6.1[1]

[1] Hippocrates, with an English translation by W. H. S. Jones, Litt. D., ST. Catharine's College, Cambridge, Vol IV, London, William Heinemann LTD, Cambridge, Massachusetts, Harvard University Press, MCMLIX.

Preface

Two years after publishing the book *Imaging in Clinical Oncology*, we have decided to rewrite Part VI on Lymphomas and present it in the form of an atlas.

This entirely new editorial work has been accomplished with the collaboration of Theodore Vassilakopoulos and Effimia Vrakidou, Department of Hematology, and Vassilis Prassopoulos, Department of Nuclear Medicine, all of whom contributed valuable criticisms and suggestions during the preparation of the book.

Several staging systems of lymphoma have been proposed and revised. Dedicated criteria have been developed for evaluating response to treatment. The most recent one is the Lugano classification system proposed for staging and response assessment. The new Lugano classification represents a consensus of experts in lymphoma diagnosis and management. These changes resulted in revision of our concepts and are reflected in the present volume.

In the past 10 years, the use of FDG PET/CT in the initial evaluation, monitoring, and follow-up of lymphoma patients has integrated new knowledge in our clinical practice. The aim of this atlas is to understand the role and limitations of FDG/PET and PET/CT in the evaluation of patients with lymphoma, in order to effectively use this imaging tool in management decisions.

Characteristic examples of FDG PET/CT imaging in patients with lymphoma are presented. The authors of this book have selected cases from the various types of Hodgkin and non-Hodgkin lymphoma in adults, children, and adolescents illustrated with FDG PET/CT imaging. After a short clinical introduction, each case is presented in a uniform fashion.

Review of the numerous cases which are presented will help the radiologists and clinicians as members of the multidisciplinary teams to understand the different aspects of lymphoma patients and become true partners in patient care.

Athens, Greece

John A. Andreou
Paris A. Kosmidis
Athanasios D. Gouliamos

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Part I

Introduction to Lymphomas

1 The 2008 WHO Classification of B-Cell Lymphomas by the Pathologist's Clinical Point of View

Dimitra S. Anagnostou

Classifications of diseases have been reasonably characterized as the language of medicine given that categorization of entities facilitates understanding among pathologists, clinicians and basic scientists while also providing at the same time a framework for the clinical practice and genesis of new data and concepts. *The World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues* (4th edition) was a collaborative project of the European Association for Haematopathology and the Society for Hematopathology [1]. The WHO classification of tumours of haematopoietic and lymphoid tissues, published as monograph in 2001 (3rd edition) [2] and updated in 2008 (4th edition) [1], represents a worldwide consensus in regard to the diagnosis of these tumours. Historically, both classifications are recognized as functional in the sense that lymphoma cells are related to the cells of the normal immune system; this constitutes a tumultuous revolution and a significant breakthrough in the field of haematopathology given that previous traditional classification schemes were based merely or entirely on morphology. Their worldwide approval relies on their clinical relevance, reproducibility and practicality, but more importantly, it depends on their solid biological background. Moreover, they have been used in clinical trials and in pathologic and epidemiological studies. Likewise, new genetic and molecular investigations rely upon these classifications.

Changes and modifications concerning the 2001 WHO classification, as well as new information, concepts and proposals, were to emerge after the publication of the 3rd, 2001, WHO classification and are presented in the updated 2008 WHO classification. This updated WHO classification incorporates new defining criteria for some diseases, biological or clinical categories or groups and provisional entities. These accomplishments resulted from new insights into clinical and laboratory research, the tremendous progression made in technology and the successful partnership of laboratory workers and clinicians. The participation in the lymphoma advisory committees of haematopathologists, clinical specialists and haematologists/oncologists led to a multiparameter approach to lymphoma diagnosis and management. Thus although histopathology remains the cornerstone in the diagnosis of lymphomas, equally critical are clinical data, immunophenotypical studies and flow cytometry in almost all circumstances, while molecular, genomic and cytogenetic techniques and, more recently, next-generation sequencing, have an increasingly important diagnostic impact.

This chapter will refer to the process and rationale for the 2008 WHO classification

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focusing selectively on those B-cell-origin diseases on which changes that have been made have had an effect in terms of the diagnostic approach and management of the patient. Moreover, in light of the fact that since 2008, new information from published studies has been presented, and new concepts have emerged, an effort will be made to selectively highlight those with clinical implications.

1.1 Principles of the Updated 2008 WHO Classification

The major principle of the 2008 WHO classification is the stratification of neoplasms primarily according to (a) their cell lineage, namely, myeloid, lymphoid B-, T-/NK- and histiocytic/dendritic cell origin, and (b) their derivation from precursor lymphoid cells, such as lymphoblastic leukaemias/lymphomas, or mature (peripheral) lymphoid cells including mature B-cell, mature T-cell and NK-cell neoplasms [3]. A normal counterpart is postulated for each neoplasm. The different types of neoplasms are listed according to (a) their clinical features; age, dissemination of disease, leukaemic picture, nodal versus extranodal localization, specific anatomic site of involvement, clinical behaviour (indolent or aggressive), history of cytotoxic or other therapies and (b) the stage of differentiation of the neoplastic cellular population, when this could be postulated. Though the 2008 WHO classification deliberately does not distinguish lymphomas by grade of malignancy, traditionally mature B-cell lymphomas composed mainly of small lymphocytes have been called low-grade lymphomas. It has been reported that, since the period of 2001–2008, the number of categories of mature B- and T-cell-origin neoplasms has almost doubled, while at the same time about 25 % more pages have been added in the updated WHO monograph (WHO) [3].

In general the updated WHO classification refined definitions of already existing diseases, identified new entities and variants, introduced new concepts and questioned the validity of some established beliefs [4, 5]. Moreover, some of the interventions that have been made are related to several discrete issues: (1) greater attention and appreciation of early or in situ lesions in the context of revealing and defining early steps in the process of evolution or transformation of a lesion, (2) introduction of new entities, (3) acceptance of age as a determining factor in the pathobiology of some diseases, (4) significant role of site-specific consequences on disease definitions, (5) initiation of provisional categories and diseases with uncertainties in definitional clinical and/or biological criteria and (6) incorporation of minor changes in terminology [6].

1.2 Aggressive B-Cell Lymphomas

Aggressive B-cell lymphomas are a diverse group of neoplasms that arise at different stages of B-cell development and by various mechanisms of transformation [7]. They include both precursor lymphoid neoplasms (B lymphoblastic leukaemia/lymphoma) and an increased number of mature B-cell neoplasms such as diffuse large B-cell lymphoma (DLBCL) with its subtypes and variants, Burkitt lymphoma (BL), mantle cell lymphoma and its blastoid variant, effusion lymphoma and B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL [7].

1.3 Diffuse Large B-Cell Lymphoma (DLBCL)

According to the updated WHO classification, diffuse large B-cell lymphoma (DLBCL) is defined as a neoplasm of large B-lymphoid cells with a nuclear size equal to or exceeding normal macrophage nuclei or more than twice the size of a normal lymphocyte. In some subtypes, such as T-cell-/histiocyte-rich large B-cell lymphoma, small non-neoplastic lymphocyte and histiocytes may outnumber the large B-cell component [1]. DLBCL is the most common type of non-Hodgkin's lymphomas (NHLs) accounting for 30 % of all adults NHLs in Western countries with 40 % encountered in extranodal sites at

stage I or II. It represents about 20 % of NHLs in children under 14 years and up to 37 % in adolescents aged 15–19 years [8]. DLBCL is one of the most heterogeneous groups of tumours and includes several types, subtypes and variants classified together on the basis of their clinical features, morphology, immunophenotype, genetic alterations and clinical behaviour. This unique clinical and biological heterogeneity of DLBCL, which is observed not only between but even within lymphoma subtypes, may explain its substantial variability to antitumour activity by novel therapeutic agents [9]. Approximately 40 % of the patients have refractory disease to chemotherapy or relapse after an initial response, while the majority of patients who relapse will succumb to the disease. It is understood that appropriate therapy presupposes stratification of the different subtypes in a clinically valid scheme.

Some DLBCLs exhibit a predilection for specific anatomic sites of presentation such as primary DLBCL of the central nervous system (CNS), primary cutaneous DLBCL leg type and primary mediastinal large B-cell lymphoma (PMBCL). However, the majority of DLBCLs without specific clinical or pathologic features are included in the group of '*not otherwise specified (NOS) lymphoma*', a histological group to which special attention will be paid in this chapter [10]. DLBCL NOS is subclassified according to the morphology (centroblastic or immunoblastic), site of primary involvement (nodal, extranodal) and clinical background (normal or compromised immunity). DLBCL can arise as de novo or in the context of progression or transformation in patients with low-grade lymphomas including chronic lymphocytic leukaemia/small-cell lymphocytic lymphoma (CLL/SCL), follicular lymphoma (FL) and marginal zone lymphoma (MZL).

1.3.1 Cell of Origin (COO) in DLBCL

The idea of distinguishing lymphomas into clinical grades (high, intermediate, low) dates back to the mid-1970s. Since then separating lymphomas has become progressively more complex; gene expression profile (GEP) has opened up a new window characterizing DLBCLs by cell of origin (COO) into biologically distinct molecular groups: (a) those with gene expression reminiscent of germinal centre B-cells (GCB-like group) (b) those with gene expression similar to activated B-cells (ACB-like group) accounting for 50 % of the cases, and (c) small number of unclassifiable cases, named 'type 3' [11]. Genes that are associated with the GCB subtype include markers of germinal centre differentiation, such as CD10 and the BCL6 gene. In the ABC type the nuclear factor kappa B (NF-kB) pathway is constitutively active, with the expression of NF-kB genes. The clinical interest of the above two subsets lies in their specific genetic alterations, different molecular signalling pathways, different clinical outcomes and potential therapeutic implications. According to gene expression signatures, most DLBCLs can be identified as prognostically favourable (GCB) or prognostically unfavourable (ABC) subtypes, both of which respond differently to therapeutic agents [12, 13]. Some authors are optimistic that eventually treatment of DLBCL will be guided by the genetic profile of tumour cells. However the wide complexity, concerning the applicability of different methods and techniques for routine use and lack of reproducibility that has been revealed by genomic studies, does not seem very encouraging at least at this point in time [1, 14]. In this context rather than applying gene expression profiling as a routine diagnostic test, several algorithms have been proposed based on immunohistochemical staining or tissue microarray analysis as purportedly useful surrogates for the classification of DLBCL subsets. In their published report from the International DLBCL Rituximab-CHOP Consortium Program Study, Visco C and colleagues introduced a new immunophenotypical algorithm called 'Visco-Young' [15]. The authors supported that using an algorithm based on the combined expression of three markers (CD10, FOXP1, BCL6) could classify DLBCL into GCB and non-GCB subgroups with high specificity and that their method can predict an outcome similar to that of GEP analysis in R-CHOP-treated patients. A short time later 17 centres from Europe, the USA and Canada successfully

tested a new method for COO assignment of DLBCL that differentiated ABC from GCB applying NanoString technology to formalin-fixed paraffin-embedded tissues in the routine diagnostic workflow [16]. However, in a study published in 2015, some investigators indicate that none of the immunohistochemical algorithms alone is sufficient to predict the outcome of patients with DLBCL especially those receiving R-CHOP therapy [17].

1.3.2 CD30-Positive DLBCL

A novel subgroup of CD3O-positive DLBCL with favourable prognosis and distinct gene expression signature was recently reported by the International DLBCL Rituximab-CHOP Consortium Program Study [18]. This study demonstrated that patients with CD30+ DLBCL had superior, 5-year, overall survival *(CD30+ 79 % versus CD30− 59 %, P = .001)* and progression-free survival *(P = .003)* in a cohort of 903 de novo DLBCL patients. CD30 was expressed in approximately 14 % of DLBCL cases. Notably, this favourable outcome was observed regardless of COO (GCB, ABC) stratification of cases [18]. Moreover, CD30 expression was predictive of superior 5-year progression-free survival within R-CHOP-treated de novo DLBCLs in a population-based study from British Columbia where the lymphoma cells were germinal centre B-cell-like (GCB) [19]. Thus CD30 immunohistochemistry was proposed as a useful prognostic marker.

1.3.3 Epstein-Barr Virus (EBV)-Positive DLBCL of the Elderly

The 2008 updated WHO classification recognized a new provisional entity defined as EBV + DLBCL of the elderly in the sense of an EBV+ monoclonal lymphoproliferative disorder arising in immunocompetent patients of advanced age encountered commonly in Asia and less frequently in North America and Europe [1]. An arbitrary cut-off of 50 years has been recommended in the updated WHO classification. EBV-positive DLBCL of the elderly is also known as age-related EBV B-cell lymphoproliferative disorder or senile EBV-associated B-cell lymphoproliferative disorders. Seventy percent of the patients presented with extranodal involvement. Morphologically the neoplastic cells may mimic Hodgkin/Reed-Sternberg cells and present marked pleomorphism [6]. Most cases demonstrate an activated ABC immunophenotype [20]. As a risk factor, EBV positivity is reported in about 10 % of DLBCL. EBV-positive DLBCL of the elderly is considered as an EBV-driven lymphoma with an aggressive clinical course in which clonality of the immunoglobulin genes and EBV can usually be detected by molecular techniques. It has been assumed that EBV+ DLBCL of the elderly might be caused by the senescence of the immune system, a phenomenon which could possibly be interpreted in the context of the normal ageing process and where the adaptive immune system is preferentially affected in contrast to the innate immunity [21]. EBV+ DLBCL may also be associated with long-standing pyothorax and may well be seen in other instances of prolonged inflammation. As EBV+ DLBCL in the elderly is still considered a provisional entity, special attention is required in distinguishing it from other EBV-related disorders including reactive lesions, EBV+ classic Hodgkin's lymphoma and the more recently described mucocutaneous ulcer.

1.3.4 The Impact of Microenvironment in DLCBCL

There is increasing evidence that in the DLBCL not only the malignant cells of the tumour but the cellular composition of the tumour microenvironment may affect survival. Similarly, stromal signatures of the tissue background and host factors may give information regarding the pathogenesis and clinical behaviour of DLBCL [7]. It is postulated that cells in the microenvironment may promote cell growth and survival or may represent part of the host response against the

tumour. Lenz and colleagues [22] demonstrated an increased survival for patients with DLBCL in these cases that the microenvironment expressed a group of genes, namely, stromal-1 genes; they identified two patterns of stromal signature: the prognostically favourable stromal-1 signature related to extracellular matrix deposition and histiocytic infiltration and the unfavourable stromal-2 signature expressing tumour blood vessel density. Additionally, genes expressed by the immune microenvironment such as TNFRSF9 and immunostains for stromal markers such as SPARC (acidic and rich in cysteine) can be used for prognostic purposes [23, 24].

1.4 Borderline Malignancies

Most lymphomas can be accurately classified as one of the currently recognized distinct disease entities. Nonetheless a group of lymphomas present with overlapping or borderline, histological, immunophenotypical, biological and clinical features between various types of lymphomas. The major overlaps occur between nodular sclerosis Hodgkin's lymphoma (cHL-NS) and primary mediastinal large B-cell lymphoma (PMBCL), lymphocyte-rich Hodgkin's lymphoma (LRcHL) and nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) and NLPHL and T-cell-/histiocyte-rich LBCL (THRLBCL). These lymphomas have been reported with different terms such as borderline lymphomas, B-cell lymphomas unclassifiable and grey zone lymphomas (GZLs). The term 'grey zone lymphoma' was firstly introduced in 1998 at the 'Workshop report on Hodgkin's disease and related diseases'. This term was further extended to lymphomas with transitional features between Burkitt lymphoma (BL) and DLBCL [25]. In the updated 2008 WHO classification, this group of lymphomas was assigned to two provisional categories called '*B-cell lymphoma unclassifiable with features intermediate between DLBCL and classical Hodgkin's lymphoma (cHL) and B-cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitt lymphoma (BL)*' [26, 27]. It is apparent that the intention of proposing such a category was to maintain the 'integrity' of the well-defined categories in the updated WHO classification and more importantly to proceed with further studies concerning their identity. This group of lymphomas comprises a heterogeneous array group of lymphoma types with variable clinical courses and a spectrum of genetic lesions. Although most grey zone lymphomas are associated with mediastinal disease, it should be noted that similar cases have also been reported in peripheral lymph nodes without mediastinal involvement [28]. The use of gene expression profiling studies further confirmed the biological relationship of the above group of diseases [28]. A representative example of this category of grey zone lymphoma is PMBCL and cHL-NS (mediastinal grey zone lymphoma (MGZL)). The use of gene expression profiling studies demonstrated that the profile of PMBCL differs from that of non-mediastinal DLBCL but is more closely related to cHL; thus this phenomenon may indicate true biological relationship between these two diseases [29]. Both, DLBCL and cHL, also show clinical, histopathological and molecular similarities and have been reported sequentially in the same patient, while in the scheme of composite lymphoma, they have been observed in the same anatomic site [30]. They both present as an anterior mediastinal mass with involvement of the thymus and/or supraclavicular lymph nodes; they affect young adults, preferentially men, and appear to have a more aggressive clinical course and poorer outcome than either PMBCL or cHL separately. Immunophenotypical findings that may play a misleading role in their diagnostic approach and in parallel justify their inclusion in the borderline category of lymphoma are [31, 32] as follows: CD30, an essential diagnostic marker for cHL, is also expressed in more than 80 % of cases of PMBCL but usually is weak and heterogeneous compared to cHL; CD15 presents in the majority of cHL (75–85 %) but may be detected in PMBCL; Reed-Sternberg-like cells and sclerosis, as part of the histological profile in PMBCL, intensify the difficulties in regard to its differential diagnosis from cHL-NS; MAL protein is expressed in 70 % of PMBCL and 10 % of cHL,

a marker which indicates their histogenetical link with the thymic asteroid medullary B-cells [25]. As a rule the neoplastic cells in PMBCL express B-cell markers; they are typically CD23 positive (70 %) and lack expression of HLA class I antigens and surface immunoglobulin (Ig). Moreover, in contrast to cHL, expression of Ig-associated transcription factors (BOB1, OCT2, PU1), is preserved. One of the major dilemmas is the interpretation of CD20 expression in cHL. As immunohistochemical standards have improved, CD20 positivity in Reed-Sternberg cells is no longer a rare finding, although its expression is usually weak and heterogeneous. Strong and uniform CD20 expression in cHL should alert the pathologist to proceed to complete immunophenotypical investigation of a particular case [33].

1.4.1 Biological Insights in Mediastinal Grey Zone Lymphoma (MGZL)

Recent studies have noticed a number of common immunophenotypical features and genetic alterations that link cHL-NS and PMBCL. There are rather relatively ample data supporting this close relationship as well as noteworthy data concerning dilemmas in the therapeutic approach of this category of lymphomas [34]. Both entities were found to be similarly positive for cyclin E (75 % and 93 %, respectively) and p63 (50 % and 53 %, respectively) performed on tissue microarray sections [28]. Classical HLs show frequent gains of regions 2p, 9p, 16p and 8q, and similar aberrations were also recognized in PMBCL [28, 35, 36]. Two of the PMBCL distinction genes, CD30 and TARC, are both expressed in the malignant Hodgkin-Reed-Sternberg (R-S) cells [37, 38]. It is intriguing that PMBCL gene signature appears to relate more to cHL gene signature than to most other DLBCLs, a finding that may support a true biological overlapping between these two diseases [29]. Similarly, analysis of methylation profiling of MGZL by Eberle and colleagues [39] showed a close epigenetic relationship between MGZL, cHL and PMBCL but in parallel demonstrated a unique epigenetic signature for MGZL, which seems to justify its classification in WHO 2008 as a separate disease entity. Moreover, in a very recent study by Steidl and colleagues [40], whole genome sequencing showed a gene fusion involving the MHC class transactivator CIITA in both cHL and PMBCL, a finding which further underscores their biological relationship.

1.5 Double-Hit (DH) B-Cell Lymphomas

Double-hit or triple-hit lymphomas are rare subtypes of lymphomas which were initially defined as mature B-cell neoplasms bearing concurrent rearrangements of the BCL2/18q21 and MYC/8q24 loci. The emergence of cumulative clinical and biological data led to the definition of double-hit lymphomas being expanded to encompass any aggressive mature B-cell neoplasm with a chromosomal breakpoint affecting MYC locus and one or more additional rearrangements recurrently associated with lymphoma, most commonly BCL2, BCL6 or CCND1 [41]. Additionally, B-cell lymphomas with more than two lymphoma-specific chromosomal abnormalities are described as triple- or quadruple-hit lymphomas [33]. MYC translocation, characteristic biological marker of BL, can also be detected, though in relatively lower frequencies, in other B-cell lymphomas such as follicular lymphoma, DLBCL and B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL. It should be mentioned that there are some essential differences between the MYC translocation in BL and in other mature B-cell lymphomas. More precisely, in BL the MYC translocation always involves one of the immunoglobulin loci and is considered a disease-initiating event which occurs in the context of a rather simple karyotype [42]. In contrast MYC translocation in other mature B-cell lymphomas, most often involving non-IG partners, are mostly found in complex karyotypes, and they possibly occur during disease progression, rather than disease initiation [42]. Gene profiling studies have shown that some

double-hit cases have a profile with features intermediate between DLBCL and *BL* [27]. In principle, this group of lymphomas is genetically defined and their incidence increases with age. All other lymphomas with a MYC breakpoint, irrespective of the presence of other aberrations, are termed 'single-hit' lymphomas. Though relatively rare, the double-hit category of lymphomas has drawn the attention of clinicians and pathologists due to their aggressive course, early relapse and resistance to treatment even when treated with R-CHOP or high-intensity chemotherapy [43]. No unifying pathologic features of double-hit lymphomas have been described in the sense that in the majority of the cases the morphological and immunophenotypical findings do not correspond with established diagnostic entities; they usually present a spectrum of pathologic features overlapping with BL, DLBCL and B lymphoblastic leukaemia/lymphoma [4]. Double-hit lymphomas have the double disadvantage of MYC (proliferation) and BCL2 (antiapoptosis). This could explain the poor prognosis and the minimal or absent response to therapy. Most patients (80–85 %) present de novo disease, while in 15–20 % of patients double-hit lymphoma develops secondarily as a result of transformation of an indolent lymphoma, most often grade 1–2 follicular lymphoma [44]. Double-hit lymphomas represent a necessary but temporary category, until more refined criteria become available.

1.6 Follicular Lymphoma (FL)

Follicular lymphoma (FL) is the second most common type of B-cell NHL comprising approximately 25 % of NHLs worldwide [45]. The vast majority of cases manifest as systemic disease involving lymph nodes. FL is characterized by the t(14;18)(q21;q32) chromosome translocation, presented in up to 80–90 % of cases. It arises from germinal centre (GC) B-cells and is composed of follicle centre (germinal centre) B-cells with morphological profile of centrocytes and large transformed cells/centroblasts. By definition, the tumour shows at least a partial follicular growth pattern. Histological grading of FL has been a matter of discussion given that this issue has been related to the outcome and the overall clinical management of the patient. The 2008 WHO classification subdivides FL into three (1,2,3) histological grades according to the relative proportion of small centrocytes and large centroblasts within the neoplastic follicles [45]. Moreover, the updated WHO classification groups grades 1 and 2 together (FL1-2), according to the presence of few centroblasts, while grade 3 (>15 % centroblasts/hpf) has been further subdivided into types A (FL3A) and B (FL3B) based on the proportion of centrocytes. More specifically centrocytes are still present in grade 3A, while in grade 3B follicles are composed entirely of large blast cells (centroblasts or immunoblasts). Additionally, the updated 2008 classification recommends that any diffuse area in an FL consisting entirely or predominantly of large blastic/transformed cells should be reported as DLBCL, as primary diagnosis, with a parallel estimate of the proportion of DLBCL and FL cell components [45].

The prognostic value of the WHO classification grading system for FL remains controversial. FL grades 1–2 and 3A have been considered as indolent, 'low-grade' lymphomas, while FL grade 3 has been connected with a more aggressive clinical behaviour [46]. However, others failed to support these findings [47]. In a recent study including the largest number of purely follicular lymphomas grade 3B (FL3B), the cytogenetic and immunohistochemical findings defined FL grade 3B as a distinct category with infrequent BCL2 and BCL6 translocations (9 % and 17 %, respectively), CD10(−) IRF4/MUM1(+) compared with FL grades 1, 2 and 3A which have shown frequent BCL2 translocations and CD10(+) IRF4/MUM1 (−) [48]. Interestingly, in a currently published study, and notably one of the few conducting studies in patients undergoing rituximab-containing therapy, overall survival and progression-free survival did not significantly differ between patients with FL grade 3 and those with FL grades 1–2 [49].

The conflicting results among various reports of FL grade 3 could be attributed to several

factors: small patient cohorts, lack of reproducibility among pathologists, differences in the diagnostic approach of the biopsy material extending from strictly morphological criteria to molecular or genetic studies and the fact that most studies were retrospective in nature. More importantly, variability in the treatment approach and the era of rituximab versus pre-rituximab and anthracycline-containing chemotherapy may have prognostic implications.

1.6.1 Follicular Lymphoma Transformation

Follicular lymphoma, initially an indolent and responsive to therapy disease, remains largely incurable [50]. A crucial event in the natural history of FL is the histological transformation to more aggressive malignancies, typically represented by a DLBCL, though transformation to lymphoblastic leukaemia/lymphoma and B-cell lymphoma unclassifiable with features between DLBCL and BL has also been documented [50] Transformation of FL to DLBCL has been reported to occur in 16–70 % of patients over time with a consensus rate of 3 % per year and is associated with a mean survival post-transformation of less than 2 years [14]. Histological transformation is usually associated with a rapidly progressive clinical course, treatment resistance and poor survival. An unequivocal definition of transformation presupposes histological confirmation of a FL diagnosis and demonstration of a clonal relationship between the original FL and the subsequently developed neoplasm. The median time from diagnosis to transformation ranges from 40 to 66 months with the earliest transformation reported at 2 months and the latest at 5 years.

The biological mechanisms which are responsible for this phenomenon of transformation are not completely understood. According to recent data on the biology of transformation of FL, it is assumed that chromosomal abnormalities associated with transformation of the disease may impair immune surveillance, activate the nuclear factor-kB pathway and deregulate P53 and B-cell transcriptor factors [51]. Moreover, whole exome sequencing and copy number analysis revealed that the dominant clone of FL and transformed FL arises by divergent evolution from a common mutated precursor cell through acquisition of distinct genetic effects [52]. Currently, Kiaii and colleagues [53] showed altered gene expression in tumour-infiltrating T-cells in FL and demonstrated that altering the immune microenvironment in FL affects overall survival and time to transformation process.

1.6.2 Variants of Follicular Lymphoma

1.6.2.1 Paediatric Follicular Lymphoma

The updated WHO classification recognizes certain distinctive clinical and genetic subtypes such as paediatric FL and primary intestinal FL.

Paediatric FL in children, even in the early stage of the disease, presents with grade 3 histology [45]. It shares many histological features with those of adult FL but lacks BCL2/IGH translocation and does not express BCL2 protein. Though paediatric FLs are of high histological grade [3], they usually present with localized disease. According to the European Intergroup for Childhood Non-Hodgkin Lymphoma and the International Berlin-Frankfurt-Münster Study Group, treatment outcome in pFL seems to be excellent with risk-adapted chemotherapy or after complete resection and ‘a watch and wait’ strategy [54].

1.6.2.2 Primary Intestinal Follicular Lymphoma

The majority of cases of primary gastrointestinal (duodenum) follicular lymphoma occur in the small intestine and most often in the duodenum, the second portion being a frequent location. Most cases are diagnosed at endoscopy. It presents as solitary or multiple small polyps and in most patients as localized disease (stage IE-IIE). Histologically the atypical follicles are found in the mucosa and submucosa, and the cellular population of the follicles consists almost entirely of centrocytes which carry the t(14;18) transloca-

tion. Duodenum FL has an indolent clinical course, and the risk of progression and dissemination is less than 5 years [6].

1.7 Early Lymphoid Lesions

The widespread use of immunohistochemistry, multicolour flow cytometry and molecular techniques on tissue biopsy samples together with the expansion in knowledge of the genetic and phenotypic alterations of specific disease entities led to the recognition of 'early' lymphoid lesions which carry some, but not all, necessary characteristics of their malignant counterparts, and their biological behaviour is uncertain [55].

Lymphoproliferative disorders associated with an indolent behaviour can be broadly separated into two groups [56]: (a) clonal proliferations that share genetic and molecular features with well-defined lymphoid neoplasms such as a monoclonal gammopathy of undefined significance (MGUS) and multiple myeloma, monoclonal B-cell lymphocytosis (MBL) and chronic lymphocytic leukaemia/small-cell lymphocytic lymphoma(CLL/SLL), follicular lymphoma in situ and follicular lymphoma(FL) and mantle cell lymphoma in situ and mantle cell lymphoma (MCL) and (b) clonal lymphoid proliferations resembling lymphomas and diagnosed as a lymphoma which do not have a counterpart among established lymphoma subtypes and are associated with an indolent clinical behaviour in the context that may represent an exaggerated antigenic response, clonal, but self-limiting [56]. This particular group includes the paediatric variants of nodal FL and nodal MZL and also the breast implant-associated anaplastic large-cell lymphoma (ALCL).

These 'early' lymphoid lesions represent an incidental finding that may be observed in asymptomatic individuals, and their biological behaviour is uncertain. Though these lesions have been reported as 'indolent', it is not clear whether they will ever progress to clinically overt lymphoma or represent self-balanced benign clonal proliferations [57, 58]. More precisely clonal lymphoid proliferations pertain to minimal infiltrates of clonal B-cells, observed and confined within apparently reactive lymphoid tissues, with some of the phenotypic and genetic features of specific B-cell lymphoma subtypes and the distinctive topographical localization of the particular lymphoma type.

Early and possibly neoplastic or pre-neoplastic lymphoid proliferations with the immunophenotypical and molecular profile of FL have been defined as 'in situ FL'. The term 'in situ neoplasia', initially applied to epithelial tumours, was later extended to the lymphoma setting. In situ FLs are characterized by the scattered presence, among their normal counterparts, of atypical lymphoid cells expressing strong BCL2+ and CD10+, positivity for CD20 and BCL6, but not IgD, and carrying t(14;18)(q32;q21) translocation. Detection of monoclonality by microdissecting BCL2+ follicles followed by polymerase chain reaction may confirm the diagnosis. The atypical lymphoid cells are strictly confined to their normal anatomic sites, usually isolated germinal centres, without any sign of invasion to the surrounding structures [59]. Cases of in situ FL have also been referred to in the 2008 WHO classification as intrafollicular neoplasia. In a similar way, in situ mantle cell lymphoma (MCL) is detected exclusively in the mantle zone of the follicles where the clonal B-cell proliferations carry some of the molecular hallmarks of its malignant counterpart such as t(11;14)(q13;q32) translocations. The concept of 'early' lymphoma is used only for cases where minimal disease extends beyond the boundaries of the follicular structures [59]. The progression of these lesions to overt lymphoma occurs rarely and may require the accumulation of additional genetic interventions. The genetic events underlying the progression from in situ FL to FL are currently unknown. Very recently Green and colleagues [60] provided evidence that IgH-BCL2 translocations and CREBBP mutations are early events during disease evolution, while MLL2 and TNFRSF14 mutations may represent late events. Moreover in addition to genetic events, microenvironmental factors may also be implicated in the process of evolution. A genomic instability has been

suggested even in the very early stages of an apparently benign lymphoid lesion.

1.8 Mantle Cell Lymphoma (MCL)

Mantle cell lymphoma is a B-cell neoplasm accounting for approximately 6 % of all NHLs. It is characterized by t(11;14)(q13;32) chromosome translocation leading to overexpression of cyclin D1 and cell cycle dysregulation in almost all cases. Cell cycle dysregulation combined with chromosomal instability and activation of cell survival mechanisms are implicated in the pathogenesis of the disease [61]. MCL presents with extensive disease and is generally composed of monomorphic small to medium-sized lymphoid cells with irregular nuclear contours. A spectrum of morphological variants of MCL with blastoid and pleomorphic histologies are also of important clinical significance [62]. To a large extent, MCL cells are CD20+, usually CD5+, FMC7+ and CD43+, but negative for CD10 and BCL6. All cases are BCL2 protein positive and in the majority of cases cyclin D1 positive. CD23 is negative or weakly positive. The cells express relatively intense surface IGM/IGD with CIgλ>CIgκ. Recently [63], the transcription factor SOX11 has been described as being expressed in most MCLs, including cyclin D1-negative cases. Aberrant phenotypes have also been described. Even though SOX11 has been characterized as a powerful tool in MCL diagnosis, its interpretation requires caution, and strict criteria should be applied given that positive SOX11 cases of DLBCL and splenic lymphoma have been reported. MCL is an aggressive disease [64]; it is considered as an incurable subtype of NHLs which, despite remissions, presents a relapse rate of approximately 50 %. Currently published studies propose that the MCL-initiated cells (MCL-ICs) are the main reason for relapse and chemoresistance of MCL. Tumour-initiated cells also referred to as cancer stem cells (CSCs) are a small fraction of cells within tumours that have been implicated in the growth, progression and relapse of several tumour subtypes [65]. It has been shown that MCL-ICs are resistant to genotoxic agents vincristine, doxorubicin and the newly approved Bruton's tyrosine kinase inhibitor (ibrutinib). A brief reference is made to a recent study with cyclin D1-positive DLBCL with IGH-CCND1 translocation and BCL6 rearrangement [66] that aimed to alert diagnosticians to its differential diagnosis from blastoid and pleomorphic variants of mantle cell lymphoma.

1.8.1 Indolent Mantle Cell Lymphoma/Leukaemia

Early studies found that leukaemic involvement in MCL was associated with aggressive disease and poor prognosis. However, a growing body of investigation studies have identified a subset of MCL, designated indolent mantle cell leukaemia, with a more indolent disease course and significantly prolonged survival, often 7–10 years, even without therapy and slow or absent clinical progression [67]. More specifically, most cases present with a leukaemic picture, absence of lymphadenopathy and hepatosplenomegaly and less frequent minimal nodal infiltration. All these cases were identified in asymptomatic patients with lymphocyte counts ranging between 4.5 and 14.2×10^9/L, and all were characterized by t(11;14) translocation. The circulating atypical cells are of small to medium size with a high nuclear to cytoplasmic ratio and slight nuclear irregularities expressing in flow cytometric analysis CD5, CD19 and CD20. However, in contrast to MCL, the indolent subtype of MCL discloses on immunohistochemistry on flow cytometry a meaningful proportion of CD23+ cells. Similarly, as opposed to typical MCL, there is a striking kappa immunoglobulin light chain restriction in most cases [68], while expression of SOX11 was absent, significantly lower than in typical MCL or only weakly expressed. Bone marrow biopsies demonstrate limited, interstitial, neoplastic infiltration. Indolent MCL presenting solely with lymphocytosis has been estimated to be just 3 % among all MCLs diagnosed during the same period.

1.9 Bone Marrow Biopsy versus FDG-PET/CT

1.9.1 General Aspects

Examination of bone marrow (BMB) has been considered as a standard procedure for the diagnosis, staging and monitoring of lymphomas. This presupposes the following [69]: Bone marrow specimens or samples, ordinarily from the posterior iliac crest, should fulfil optional quantitative and qualitative standards; the pathology lab should respond to the current technological advances; pathologists are specialized to the field of haematopathology and collaboration with the clinician is a must. In most cases morphological and immunophenotypical findings allow a definitive diagnosis. However, selected cases are supplemented with cytogenetics and interphase fluorescence in situ hybridization (FISH). Concerning treatment response measurements, which target the detection of a limited number of tumour cells, namely, minimal residual disease (MRD), molecular tests are designed to detect specific translocations or specific DNA sequence of the antigen receptor of a particular lymphoma [69] A monolateral long (3–4 cm) cylinder, ordinarily taken from the posterior iliac crest, is considered sufficient for an accurate diagnosis and sufficiently reliable to detect focal BM involvement in patients particularly in those with Hodgkin's disease.

1.9.2 Relevance of BMB versus FDG-PET/CT in Lymphomas

Bone marrow biopsy has been a reference standard procedure in lymphoma staging, and marrow histology has been incorporated into the new National Comprehensive Cancer Network International Prognostic Index. However, through the years positron emission tomography (PET) using fluorodeoxyglucose (FDG) with computed tomography (CT) has become an established modality for staging newly diagnosed lymphomas particularly FDG-avid lymphomas, nodal and extranodal, including bone marrow assessment [70]. The major advantages imputed to FDG-PET/CT over BMB by its supporters include: noninvasiveness of the procedure, absence of sampling errors given that BMB assesses only a very small portion of the entire bone marrow and thus focal BM involvement can be missed by BMB, its capacity to distinguish viable tumour from scars and fibrosis and less time consuming considering the preparation procedures needed for fixation and decalcification stages [71]. An impressive number of articles presenting new data on the role of FDG-PET/CT in the evaluation of BM in HL and DLBCL, including lately follicular and paediatric lymphomas, have seen the light in recent publications [72]. Hence, the high sensitivity of PET/CT for BM involvement has recently prompted many authors to question the continued use of BMB in some histologies. The response was given recently at the 12th International Conference on Malignant Lymphoma in Lugano, Switzerland, in June 2013 where the initially developed criteria for staging and response for HLs and NHLs were revised [73]. As a result, establishment of modernized recommendations for initial evaluation, staging and response assessment in patients with HLs and NHLs was recorded. Additionally, FDG-PET/CT was formally incorporated into standard staging for FDG-avid lymphomas, whereas CT scan was indicated for non-avid histologies. In summary the conclusions drawn at this conference concerning BMB are the following [73]: (1) If a PET/CT is performed, a BMB is no longer indicated for routine staging of HL, (2) a BMB is only needed for DLBCL in the event that PET is negative and identifying a discordant histology in DLBCL lymphomas is important for patient management. Moreover, a 2.5 cm unilateral BMB was recommended along with immunohistochemistry and flow cytometry, whereas a preference to excisional biopsy was proposed. It is intriguing that in April 2015, a letter to the editor by Adams JA and Kwee TC, headed 'Do Not Abandon the Bone Marrow Biopsy Yet in DLBCL', expressed the opinion that whether FDG-PET/CT can replace BMB has yet to be proven both from a diagnostic and prognostic perspective [74]. Interestingly, in a

large international cohort study to better define the use of PET in DLBCL, Cerci and colleagues [75] concluded that neither BM histology nor PET alone is a reliable indicator of poor-risk marrow disease and that the most consistent indicator is marrow involvement identified by both PET scan and histology. It should be clarified that in regard to other lymphoma histologies, the existing data is considered insufficient to permit similar changes in practice as in HL and DLBCL HL. Thus BMB continues to be recommended for staging all other lymphoma histologies providing that it would affect treatment.

1.9.3 Prognostic Impact of BM Histological Subtypes in DLBCL

Approximately 10–25 % of patients with DLBCL exhibit BM involvement at diagnosis. BM histology in DLBCL has been incorporated in the National Comprehensive Cancer Network. In many patients with DLBCL, BMB presents histology similar (concordant) to that of the primary site of involvement, i.e. infiltration with large B-cells. In 40–72 % of patients the histology at the time of staging is discordant in the sense that BM in contrast to lymph node histology is infiltrated by small B-lymphocytes [76]. In this context it is presumed that the DLBCL may have developed as a transformation of an occult small B-cell lymphoma or alternatively two unrelated lymphomas are present [77]. A recently published large retrospective population-based study represents the first comprehensive analysis [76] on the prognostic impact of BM involvement in 795 unselected patients with DLBCL treated with R-CHOP, based on the histological type of the infiltration, concordant or discordant. It was demonstrated in this study that the outcomes of patients with concordant BM involvement were inferior to the outcomes of those with no marrow involvement or those with discordant lymphoma. More precisely, the negative prognostic impact of discordant BM involvement affects progression-free survival (PFS), but not overall survival (OS), and it is adequately represented by the International Prognostic Index (IPI) score. In contrast, concordant BM involvement is characterized by a negative impact both on the PFI and OS independently of the IPI. Concordant BM involvement was associated with poor performance status with elevated lactate dehydrogenase, advanced stage, an increased number of extranodal sites and older age patients. Nonetheless the effects of discordant lymphomas, in regard to patient's outcome, are controversial; some studies claim that discordant BM involvement does not affect patient's outcome, while others support a higher rate of delayed relapses.

1.10 Hodgkin's Lymphoma (HL)

Hodgkin's lymphoma is a lymphoid tumour that represents about 1 % of all de novo neoplasms occurring every year worldwide. In the 1990s it was recognized that the neoplastic cells of Hodgkin's lymphoma (originally designated as disease) were of B-cell lineage. The diagnostic criteria for Hodgkin's lymphoma (HL) have been refined in the 3rd and 4th editions of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues [78]. Based on clinical, biological and histological findings and the microenvironmental surroundings, it has been shown that HL consists of two distinct disease entities [79, 80], namely, nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) and classical Hodgkin's lymphoma (cHL) with the latter comprising the following histological subtypes: lymphocyte rich (LRHL), nodular sclerosis (NSHL), mixed cellularity (MCHL) and lymphocyte depleted (LDHL). There are basic distinctions between NLPHL and cHL in clinical presentation, behaviour, morphology, phenotype, molecular profile and composition of the cellular background. In this context NLPHL deserves special attention, and thus, in the interest of space, it will be given a more generous reference comparatively to cHL and its subtypes. NLPHL represents 4–5 % of all HL cases and histologically may present several distinct immunoarchitectural patterns. However, according to the current WHO

classification, at least a partial nodular pattern is required for its diagnosis. NLPHL, in contrast to cHL, has no relationship with EBV infection, patients are predominantly middle-aged males and the disease is localized (stages I–II in 70–80 %), usually affecting single cervical, axillary or inguinal nodes. Many patients may present with very large solitary masses with no other signs or symptoms of lymphoma. Bone marrow and mediastinum are rarely involved. The disease shows a very indolent course with prolonged disease-free period and high rates of relapses. The characteristic cell for the disease is the lymphocyte-predominant (LP) cell [79] which is a large mononuclear cell with a polylobular appearance, finely dispersed chromatin and multiple, small, basophilic nucleoli, while it only occasionally may show features of Hodgkin's-Reed-Sternberg cell. LP cell presents a germinal centre (GC) phenotypic profile with expression of GC markers together with expression of transcriptor factors such as OCT-2 and BOB-1. Lymphocyte-predominant cells are positive for the epithelial membrane antigen (EMA), and in contrast to cHL, they are CD15 negative and generally lack CD30, although a small subset may be CD30 positive since CD30 is an activation marker. Heavy and light chains of immunoglobulins are frequently expressed, and in particular IgD positivity identifies a subgroup of cases with peculiar epidemiological, phenotypic and clinical features [81]. Occasionally, NLPHL may be confused with an atypical form of follicular hyperplasia, namely, follicular hyperplasia with progressive transformation of germinal centres (PTGC) [79, 82]. This lesion is observed particularly in children and young adults and may precede, concur or follow NLPHL. Its pre-neoplastic nature has not been confirmed, though it seems to be associated with a slightly higher risk of evolving to NLPHL. Notably, BM involvement in NLPHL may not be strictly related to the disease stage, as BM involvement has also been recorded even in stage IA patients, a situation that is associated with aggressive clinical behaviour.

Progression of NLPHL, commonly to a clone-related DLBCL, occurs between 6 months and 24 years after NLPHL diagnosis. In the past 20 years, simultaneous or secondary transformation of NLPHL into DLBCL has been reported in up to 30 % of cases [83]. With the exception of one study [84], several authors have suggested that patients with NLPHL and DLBCL, simultaneously presented in the same lymph node, will have a favourable clinical outcome [83, 85]. Additionally, patients with sequential transformation have shown a nonsignificant tendency towards worse overall and progression-free survival compared to patients with simultaneous presentation of NLPHL and DLBCL. The morphological and phenotypic features of the DLBCL deriving from NLPHL are heterogeneous, a phenomenon that could possibly explain the different mechanisms involved in the transformation process [83]. Recently, germline mutations of the *NPAT* gene have been recognized as a risk factor for NLPHL in the Finnish population [86], and there are also reports about a high familial risk in first-degree relatives of NLPHL patients [87].

Classical Hodgkin's lymphoma [79] shows a bimodal age distribution. It is characterized by dissemination and presents variable correlation with EBV in 30–40 % of cases, depending on the histotype of the disease. All histological subtypes of cHL are characterized by the presence of Hodgkin's and Reed-Sternberg cells and variants. They all express the same phenotypic profile: CD45−, CD30+, CD15+/− (positivity in 75–80 % of cases), CD79−, CD20− (variably + in 20 %), IRF4+, EMA−, BCL6−, PAX5/BSAP+ (with a few exceptions) and Ig-transcription factor. The diagnosis of cHL is based on the identification of the characteristic for the disease neoplastic cellular component (lacunar cells, mononuclear Hodgkin's and multinucleated Hodgkin's-Reed-Sternberg cell) and the tissue background (inflammatory milieu, collagen bands, the proportion of small reactive lymphocytes). HRS cells even diagnostic are not exclusive for HL. Though the neoplastic components in cHL, as in NLPHL, are related to germinal centre B-cells, the former display high load of somatic mutations becoming subsequently stable, while in the latter ongoing mutations are detected in about half of the cases [88].

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