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Commentary

Aggressive B-cell lymphomas with a primary bone marrow presentation

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Introduction

Aggressive B-cell lymphomas with a primary bone marrow (BM) presentation constitute a heterogeneous group of entities that present either as the first diagnostic site in the BM of well-defined World Health Organization (WHO) entities, which might also involve other tissues (most commonly lymph nodes), or diseases that typically, and sometimes exclusively, involve the BM. This latter category is a mix of one WHO-defined entity, namely intravascular large B-cell lymphoma (IV-LBCL), and diseases whose diagnostic criteria (sometimes contrasting between studies) have been proposed in the literature but not yet officially accepted in the WHO classification. In contrast to small B-cell lymphomas, each category of aggressive lymphoma primary to the BM is in itself relatively rare, which adds to the diagnostic challenge already presented by the intrinsic difficulties of this type of biopsy. For some of them, such as primary diffuse large B-cell lymphoma (DLBCL) of the BM (PBM-DLBCL), the BM, is by definition, the primary diagnostic site; other entities might be habitually diagnosed in other sites, and might present difficulties when the BM is the only available material. Subclassification of aggressive B-cell lymphomas in the BM poses specific problems related to the site, such as separation from B-cell acute lymphoblastic leukaemia (B-ALL)/B-cell lymphoblastic lymphoma (B-LBL) and the definition of high-grade transformation of indolent B-cell lymphoma.

For these reasons, aggressive B-cell lymphomas with a primary BM presentation were chosen as one of the topics for the BM workshop of the 19th meeting of the European Association for Haematopathology/Society of Hematopathology (EAHP/SH), which took place in Edinburgh in September 2018. The general theme of the workshop was lymphoid proliferations with primary presentation in the BM (excluding small B-cell lymphomas), and separate papers dealing with peripheral T-cell lymphomas with a primary BM presentation and Hodgkin lymphoma with a primary BM presentation can be found in this journal.

This article presents the seminal findings of the workshop session dealing with mature aggressive B-cell lymphomas, which comprised the WHO categories Burkitt's Lymphoma BL, high-grade B-cell lymphoma (HG-BCL) with *MYC* and *bcl-2* and/or *bcl-6* rearrangements, and HG-BCL not otherwise specified (NOS), as well as the spectrum of PBM-DLBCL and variants, including IV-LCBL and the recently proposed large B-cell lymphoma presenting in the BM, spleen, and liver (BSL-LBCL), primary bone DLBCL (PB-DLBCL), and other cases of unusual primary presentation in the BM, such as Epstein–Barr virus-positive DLBCL or anaplastic lymphoma kinase-positive DLBCL. Furthermore, several cases highlighted diagnostic problem zones, such as double-hit (DH) lymphomas with terminal deoxynucleotidyl transferase (TdT) expression or the difficult distinction between T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) when presenting primarily in the BM. In total, 44 cases were received for this session of the workshop.

BL

BL is a well-defined aggressive B-cell lymphoma. It is the second most common B-cell neoplasm in children, following B-ALL/B-LBL. BL is characterised by medium-sized blasts with multiple small nucleoli and basophilic cytoplasm, and a characteristic immunophenotype with expression of the germinal centre markers CD10 and bcl-6; usually, but not always, lack of bcl-2 expression; variable expression of interferon regulatory factor 4 (IRF4)/multiple myeloma 1 (MUM1) in a subset of cases; and a very high proliferation rate. Additional markers that are useful for BL diagnosis when used in the right context are CD38, which is positive in most BLs, but also in a subset of HG-BCLs with DH/triple-hit (TH), but negative in Burkitt-like lymphoma with 11q aberration, and CD44, which is negative in most BLs.[1–3]

Genotypically, BL shows a translocation of *MYC* with the IG heavy or, less frequently, IG light chain genes, a simple karyotype, and a mutational spectrum distinct from that of other aggressive B-cell lymphomas, with common alterations of *ID3*, *TCF3*, *CCND3*,[4] and others.[5] BM infiltration is common,

occurring in 30% of patients, so the BM is frequently the first site of biopsy. Some cases present with BM and peripheral blood (PB) involvement only, and these are referred to as Burkitt leukaemia. Separation from B-ALL/B-LBL relies on a mature B-cell phenotype with an absence of TdT expression, but may be difficult in some cases. Cases with *IG–MYC* translocation and a precursor B-cell phenotype show a distinct spectrum of molecular alterations from conventional BL.[6]

Three cases of BL/leukaemia were submitted to the workshop, all showing some phenotypic or genetic abnormalities (Figure 1). The two cases (248 and 469) classified as Burkitt leukaemia lacked peripheral lymphadenopathy, and showed a packed BM (Figure 1A–C). Case 469 in a 7-year-old female showed leucocytosis of 41.4×10^9/l with 54% blasts and an aberrant phenotype with lack of surface and cytoplasmic IG and CD20 and bcl-6 negativity as determined with immunohistochemistry, but with CD20 positivity as determined with flow cytometry, indicating a more immature phenotype that is observed in rare cases of otherwise typical BL/leukaemia. Case 248 in a 34-year-old male showed rare circulating blasts and a classic BL immunophenotype. Both cases, however, lacked CD34 and TdT, and had a *MYC* translocation. Case 385 showed a typical immunophenotype (CD10+, bcl-6+, MYC+, bcl-2−), but fluorescence *in-situ* hybridisation (FISH) failed to show a *MYC* translocation, or 11q aberrations (Figure 1D). Molecular studies, however, revealed *ID3*, *CCND3* and *MYC* mutations, which are characteristic for BL. Three additional workshop cases (309, 361, and 412) occurring in a 52-year-old male, a 4-year-old male and a 67-year-old female, respectively, harboured an *IGH–MYC* translocation and lacked *bcl-2* or *bcl-6* translocations, but were classified as B-ALL because of an immature phenotype with TdT expression. These three cases, two of which had leukaemic presentations, highlight the occasional difficulty in separating BL from B-ALL.[7]

Finally, one case (266) with morphological features suggestive of BL showed a *MYC* translocation as determined with karyotyping and FISH, but had a T-cell immunophenotype and was interpreted as T-lymphoblastic lymphoma. This case, seen in a 5-month-old boy, showed a translocation between *MYC* and *TRA*, and had morphological, immunophenotypic and genetic features similar to those in a prior case report of a 4-year-old girl.[8] The prior case responded well to high-risk acute lymphoblastic leukaemia therapy followed by haematopoietic stem cell transplantation.

HG-BCL with *MYC* and *bcl-2* and/or *bcl-6* rearrangements and HG-BCL NOS

HG-BCLs have recently been defined as a separate entity in the 2017 update of the WHO classification, and replace the previous provisional category of HG-BCL, unclassifiable, intermediate between DLBCL and BL of the 2008 WHO classification.[4,9] They comprise two subgroups of cases of aggressive B-cell lymphoma, which should not be classified as DLBCL or BL, respectively (Table 1). The first group, containing approximately two-thirds of the cases, is characterised by the presence of a *MYC* translocation in combination with a *bcl-2* and/or a *bcl-6* translocation. Morphologically, they can show DLBCL morphology, BL-like morphology, or intermediate features between BL and DLBCL, or have a blastoid appearance.[10–12] Rare cases of follicular lymphoma (FL) and B-LBL with DH/TH are excluded. Approximately half of the cases with *MYC*/*bcl-2* DH show a history of FL, and thus represent high-grade transformation. With the exception of *MYC*/*bcl-6* DH cases, most cases show a germinal centre B-like (GCB) subtype according to the Hans algorithm.[13] The second group comprises HG-BCL NOS. It contains cases lacking DH/TH, that have either a morphology that could be defined as intermediate between BL and DLBCL NOS, or blastoid features. The diagnostic separation from DLBCL thus relies on purely morphological criteria, and is probably subject to significant interobserver variability, as also noted in this workshop. Without touching upon the extensive and, in part, controversial literature on the prognostic relevance, it can be stated that many cases of HG-BCL of both categories show advanced disease with aggressive behaviour and a poor prognosis. BM involvement is common, and so the BM is frequently the site of primary diagnosis.

A total of nine cases of HG-BCL with DH/TH and a mature phenotype were submitted to the workshop, including five cases with *MYC*/*bcl-2* DH, two cases with *MYC*/*bcl-6* DH, and two TH cases (Figure 2), including case 453 with an unusual complex three-way translocation involving *MYC*, *bcl-2*, and *bcl-6*, and a non-GCB phenotype. Patients usually presented with PB cytopenia and B-symptoms, two cases had a clonally related FL or DLBCL (without DH) in the lymph node biopsy, and two cases showed leukaemic PB involvement. Two cases showed low proliferation despite the presence of DH, which in case 416 was potentially due to steroid treatment before BM biopsy. In case 460, the morphology was difficult to assess, and the panel felt unable to assign it to a specific category. With the exception of case 453 with the three-

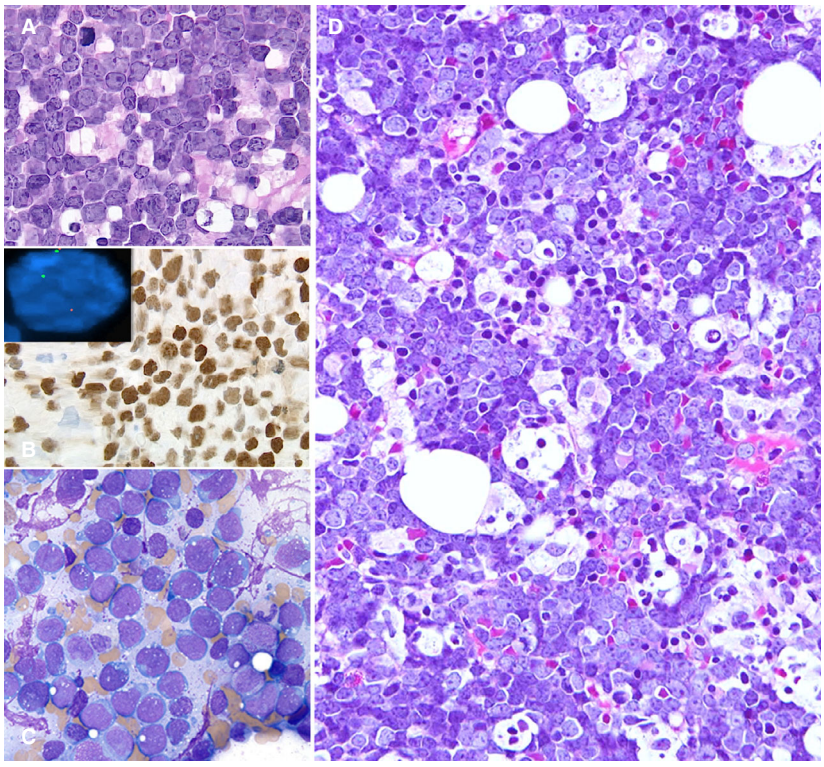

Figure 1. Burkitt lymphoma (BL)/leukaemia. **A,B,** The bone marrow (BM) trephine biopsy of case 248 shows tightly packed, medium-sized cells with a high mitotic rate (**A**) and with strong *MYC* expression (**B**) and the presence of a *MYC* break (**B,** insert). **C,** The BM aspirate of case 469 (Burkitt leukaemia) shows the typical BL cytology with blast cells with deeply basophilic, vacuolated cytoplasm, a high nuclear/cytoplasmic ratio, and medium-sized nucleoli. **D,** The BM trephine biopsy of case 385 classified as BL despite lacking *MYC* translocation as determined with fluorescence *in-situ* hybridisation with tightly packed, medium-sized blasts with many mitotic figures and starry sky macrophages.

way translocation (Figure 2B), all cases showed a GCB phenotype according to the Hans algorithm, with 279 showing a small subpopulation of TdT-positive cells (Figure 2A). Seven additional cases with predominantly TdT-positive cells with DH/TH were also submitted to the workshop (185, 235, 267, 273, 287, 411, and 443). Two of these presented with TH, and the other five had both *MYC* and *bcl-2* rearrangements. On the basis of strong expression of TdT, these were classified as B-ALL/B-LBL rather than HG-BCL. Of those, five of six with available karyotype or array comparative genomic hybridisation data showed a complex karyotype in addition to the *MYC* and the other *bcl-2* and/or *bcl-6* translocations. Although the recent update of the WHO classification clearly states that these cases have to be classified as B-ALL/B-LBL, the precise classification of these rare cases remains a matter of debate, and further research is needed.[14]

Six cases were assigned to the HG-BCL NOS category, but the panel favoured this diagnosis in only four cases, because the morphology was, in part difficult, to assess, and the current morphological definition leaves some room for interpretation. Two cases tentatively assigned to the HG-BCL NOS group, including case 542 with coexpression of CD5, showed massive leukaemic PB involvement, raising the differential diagnosis of B-cell prolymphocytic leukaemia (B-PLL). Leukaemic blastoid mantle cell lymphoma was ruled out by negativity for cyclin D1 and SOX11. The panel emphasised that, in addition to morphological features consistent with HG-BCL NOS, stains for cyclin D1, TdT, SOX11, CD5 and others are required to rule out other aggressive B-cell lymphomas.

A disorder that is relevant to consider in the differential diagnosis of HG-BCL NOS and BL is Burkitt-like lymphoma with 11q aberration, which was introduced as a provisional entity in the 2016 update of the WHO classification.[4,15] No such case was submitted to the workshop, but the cases of HG-BCL NOS had not been studied systematically for this aberration.

PBM-DLBCL, IV-LBCL, and large B-cell lymphoma presenting in the BM, spleen, and liver

Large B-cell lymphomas with primary presentation in the BM frequently possess clinicopathological features which set them apart from conventional DLBCL NOS arising at extramedullary sites. In the workshop, this group included several challenging cases, which were difficult to assign to a diagnostic category, owing to subjective interpretation of criteria for well-defined WHO entities, as well as inconsistent published

Table 1. Differential diagnosis of Burkitt lymphoma (BL), double-hit (DH)/triple-hit (TH) high-grade B-cell lymphoma (HG-BCL), and HG-BCL not otherwise specified (NOS)

	Morphology	Phenotype	Genetics	Comments
Burkitt lymphoma	Monotonous proliferation of medium-sized blasts with scant basophilic cytoplasm, small nucleoli, and a starry sky pattern	pan-B+, CD10+, bcl-6+, MUM1−/+, MYC+, bcl-2−/(+), TdT, Ki67 100, CD44−, CD38+	Simple karyotype with *MYC* rearrangement, 85% t (8;14)/*MYC*/IGH, the rest t (2;8) or t(8;22) with *MYC*/IGK or *MYC*/IGL. *TCF3*, *ID3*, *CCND3* and *TP53* mutations	Mostly children and young adults, may present as leukaemia. TdT + cases with *MYC*-R are classified as B-ALL/B-LBL *MYC*-R-negative cases with 11q alterations excluded
HG-BCL with DH/TH	BCL-U, blastoid or DLBCL morphology FL with DH/TH and B-precursor neoplasms excluded	With the exception of MYC/bcl-6 DH, mostly of the GCB phenotype. MYC frequently strongly positive, bcl-2 positive in MYC/bcl-2 DH, TdT−, MIB1 variable	*MYC*/*bcl-2* DH 58–75% *MYC*/*bcl-6* DH 5–15% TH 10–20% Genetic alterations other than translocations and other translocations do not count as DH/TH	Approximately half of *MYC*/*bcl-2* DH lymphomas represent transformation of previous FL *MYC*-Rs with non-IG partner show a better prognosis (shown for DLBCL morphology)
HG-BCL NOS	BL-like, BCL-U or blastoid morphology	GCB> non-GCB phenotype, MYC+/−, MIB1 variable	Single *MYC*-R in 20–35% of cases 11q alterations excluded	Stains for cyclin D1, SOX11 and TdT required to exclude blastoid MCL and B-ALL/B-LBL

BCL-U, intermediate between DLBCL and BL; B-ALL/B-LBL, B-cell lymphoblastic leukaemia/lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; GGCB, germinal centre B-like; MCL, mantle cell lymphoma; MUM1, multiple myeloma 1; MYC-R, *MYC* rearrangement; TdT, terminal deoxynucleotidyl transferase.

criteria for novel entities proposed in the literature. As an example, the amount of tolerated extravascular growth allowed for a diagnosis of IV-LBCL is unclear, and could switch a diagnosis to DLBCL NOS. During the workshop, each case was assigned to a WHO-defined category, which included DLBCL NOS, IV-LBCL and HG-BCL with *MYC* and *bcl-2* and/or *bcl-6* rearrangements. However, considering the interesting scientific data supporting the creation of novel categories, we also discussed whether a case could fulfil the published criteria for literature-proposed entities.

The 2017 WHO classification of haematological neoplasms provides no official definition of PBM-DLBCL, which would currently be classified as DLBCL NOS according to current WHO definitions. Several published definitions consist of a spectrum comprising 'purist' approaches, such as the one adopted by the International Lymphoma Study Group,[16,17] not allowing any localisation beyond the BM, and more 'relaxed' definitions, for instance allowing for hepatosplenomegaly[18] or sometimes even for local lymphadenopathy.[19] More complexity is added by the distinction between PBM-DLBCL and PB-DLBCL, whereby the latter (recognised as an entity in the WHO classification of tumours of soft tissues and bone[20]) presents with single or multiple lytic lesions (involving, *de facto*, also the BM) and the former presents with disseminated BM disease in the absence of osteolysis, even at the microscopic level. The reported patterns of BM involvement by PBM-DLBCL are nodular and/or diffuse or, rarely, intrasinusoidal.[16,21] From the best characterised series, it seems that almost equal proportions of GCB and non-GCB cases are present,[16] although a recent analysis of published cases reported CD10 expression in an overall minimal percentage of cases, whereas most of them expressed IRF4/MUM1,[17] with most cases therefore being classified as non-GCB according to the Hans algorithm.[22]

IV-LBCL is defined, according to the WHO, as an extranodal lymphoma characterised by selective growth within the lumina of vessels, with the exception of larger arteries and veins.[23] Currently, two subtypes are recognised: the 'classic' or 'Western' type, and an Asian or 'Eastern' type. The two forms differ in clinical presentation, with the Western type showing mainly neurological and cutaneous symptoms, and the Eastern type presenting with fever of

unknown origin, haemophagocytic syndrome (HPS), multiorgan failure, pancytopenia, and hepatosplenomegaly. To fulfil the definition, the neoplastic cells should primarily be located intravascularly, but minor extravascular spilling is allowed, and in the central nervous system (CNS) even mass-forming lesions are compatible with this diagnosis. Neoplastic cells can be detected in the PB in 4–24% of cases.[24] In the BM, spleen and liver sinusoidal involvement occurs. Recently, a wider morphological spectrum of BM involvement, also including cases with interstitial or diffuse involvement besides intrasinusoidal growth, has been described,[24] again illustrating the varying definitions used in the literature. IV-LBCL lacks a

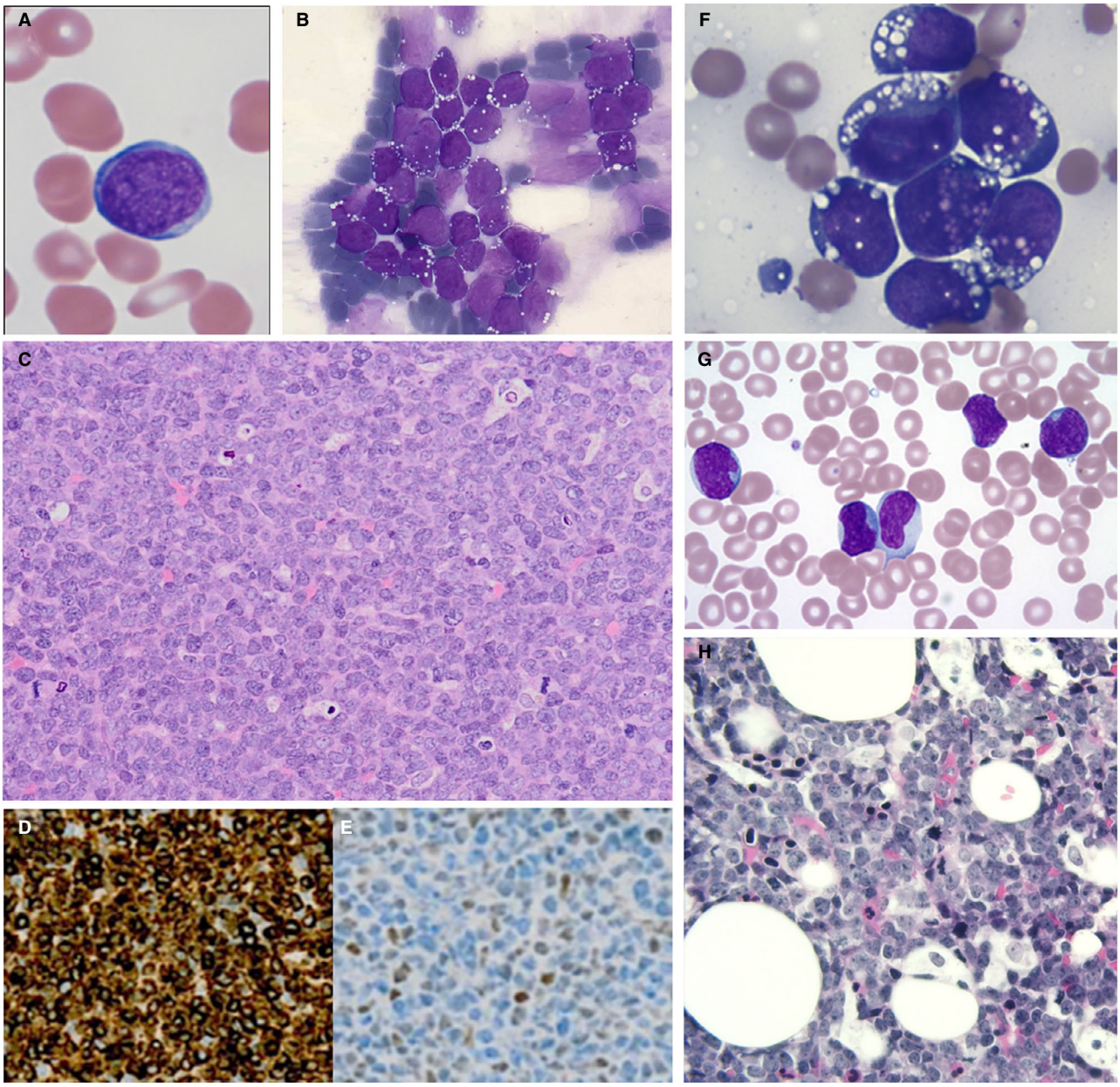

Figure 2. The spectrum of high-grade B-cell lymphoma (HG-BCL) with double-hit/triple-hit (DH/TH) and HG-BCL not otherwise specified (NOS). **A,** Circulating blasts with a high nuclear/cytoplasmic ratio (case 279) **B,** Bone marrow (BM) aspirate and biopsy with medium-sized blasts with cytoplasmic vacuolation (case 453). **C,** *MYC*/*bcl-2* DH lymphoma (case 116) with a monomorphic blastoid appearance. **D,E,** Strong bcl-2 staining (**D**) and heterogeneous MYC expression (**E**) in HG-BCL NOS. **F,G,** Cytology (**F**, case 286) and BM trephine (**H**, case 538) appearance of two cases classified as HG-BCL NOS by both submitters and the workshop panel.

specific immunophenotype, but CD5 is expressed in approximately 40–50% of cases, and the great majority of cases show a non-GCB phenotype, and CD10 expression is very rare. The subclassification into GCB and non-GCB subtypes, however, is not deemed to be necessary in this entity by the current WHO classification.

The expression of CD5 brings *de-novo* CD5-positive DLBCL (i.e. excluding cases evolving from another CD5-positive lymphoma) into the differential diagnosis. Several studies independently showed that CD5-positive DLBCL might constitute a distinct clinicopathological entity, with a poorer prognosis, more frequent extranodal and CNS involvement, poor performance status, and a poorer response to rituximab-containing regimens, as well as a predominance of the non-GCB immunophenotype.[4,25–27] Interestingly, features of CD5-positive DLBCL also include a significantly higher frequency of BM infiltration.[27] Unfortunately, the specific morphological patterns of BM infiltration were not described; immunohistochemically, these cases also showed a higher percentage of non-GCB cases, frequent FOXP1 expression, and significantly higher bcl-2 expression.

Recently, a subtype of DLBCL primarily involving the BM, spleen and liver (BSL-LBCL) was proposed.[28,29] This lymphoma subtype would, in most instances, be classified as DLBCL NOS according to the WHO classification, with a minority of cases being classified as DH/TH high-grade large B-cell lymphoma and some cases being borderline with IV-LBCL. Its clinical features seem strikingly similar to those of the Eastern type of IV-LBCL, presenting with B symptoms, cytopenia, hepatosplenomegaly, haemophagocytosis, and occasional PB involvement. On fluorodeoxyglucose-positron emission tomography (FDG-PET), the BM showed a diffuse increase in tracer uptake, mainly in the central skeleton, accompanied by diffuse uptake in the spleen and often in the liver. The differential diagnosis between BSL-LBCL and IV-LBCL is based on the different pattern of BM involvement, characterised by diffuse or interstitial infiltration, although intrasinusoidal involvement was also occasionally demonstrated, sometimes with concurrent CD5 expression. As a distinctive feature, a higher percentage of cases with PB involvement was reported for BSL-LBCL than for IV-LBCL. Genetically, a high frequency of *bcl-6* rearrangements, typically non-*IG–bcl-6* translocations, as well as copy gains of *bcl-6*, *bcl-2* and *MYC*[28] and other recurrent abnormalities involving the 19q13 region[29] have been reported.

Overall, PBM-DLBCL presents a spectrum of overlapping disorders with rather indistinct borders (Table 2); many cases from reported series might be easily reclassified as a different subtype, according to the interpretation of the proposed criteria. From the point of view of the WHO classification, most cases should be classified as either IV-LBCL or DLBCL NOS, usually of the non-GCB type, as the other proposed variants (PBM-LBCL, BSL-LBCL, and CD5-positive DLBCL) are not defined WHO entities. Even this dichotomous separation, however, has grey zones, as shown by the workshop cases.

Two cases (442 and 481) satisfied the strictest proposed criteria for PBM-DLBCL,[16] i.e. no lymphadenopathy, no hepatosplenomegaly, and no lytic lesions. Case 442 presented with concurrent, probably reactive, dysplastic features of haematopoiesis. Clinically, both cases presented with fever of a few weeks' duration and B-symptoms (weight loss, night sweats, or malaise), anaemia and thrombocytopenia, and a high lactate dehydrogenase level. Morphologically, case 442 presented with a patchy irregular infiltrate of large cells (20% of total cellularity); for case 481, two BM biopsies were provided, one with subtle interstitial/intrasinusoidal involvement, and a second with massive diffuse involvement (Figure 3A, B). Immunophenotypically they showed a mature B-cell phenotype and a non-GCB subtype according to the Hans algorithm; case 442 also showed CD5 expression.

Three cases (215, 291, and 337) satisfied the criteria for IV-LBCL.[23] Case 291 presented with fever and pancytopenia, and case 337 also showed syncope, progressive weakness, and deteriorating mental status, whereas case 215 showed a milder clinical picture, with only weakness, anaemia, and slight thrombocytopenia. None of the cases presented with HPS. Morphologically, only case 215 showed a pure intravascular/intrasinusoidal pattern (Figure 3C), and both case 291 and case 337 showed some leakage of neoplastic cells in the perivascular area (Figure 3D). Case 291 also showed a pure intravascular pattern in the stomach (resected in order to remove a gastrointestinal stromal tumour). All cases showed a B-cell phenotype with coexpression of CD5; case 291 expressed CD10 and was therefore classifiable as being of the 'GCB' type, whereas the remaining two cases were of the 'non-GCB' type; it should be noted, however, that subtyping is not recommended for IV-LBCL according to the WHO classification.

Two cases (229 and 300) presented with features of BSL-LBCL,[28,29] although case 300 also showed, on FDG-PET, some uptake in lymph nodes, which makes it fall outside the usual spectrum for this disease. However, as this is not a recognised WHO entity, the boundaries of this DLBCL subtype are not well

Table 2. Clinicopathological spectrum of primary diffuse large B-cell lymphoma (DLBCL) of the bone marrow (BM) (PBM-DLBCL), compared to DLBCL not otherwise specified (NOS), CD5-positive DLBCL, intravascular large B-cell lymphoma (IV-LBCL) and large B-cell lymphoma with involvement of the BM, spleen, and liver (BSL-LBCL)

	DLBCL NOS	PBM-DLBCL	IV-LBCL-W	IV-LBCL-E	BSL-LBCL
Predominant ethnicity	Caucasian	NR	Caucasian	Asian	Asian
Fever	+/−	−/+	−	+	+
Haematophagocytosis	−	−	−	+	+
Pancytopenia	+/−	−/+	−	+	+
Peripheral blood involvement	−/+	−	+/−	+/−	+
Spleen enlargement	+/−	−	−	+	+
Liver enlargement	−/+	−	−	+	+
Lymph node enlargement	+	−	−	−	−
CNS involvement	−/+	−	+	−	−
Lytic bone lesions	−	−	−	−	−
Skin involvement	−	−	+	+/−	−
Intrasinusoidal/intravascular	−	−/+	+	+	−/+
Interstitial	−/+	−	+/−	+/−	+/−
Nodular	−/+	+/−	−	−	−/+
Diffuse	+	+/−	−	−	+/−
CD5	−/+	−	+/−	+/−	+/−
Hans classifier	GCB≈non-GCB	GCB≈non-GCB	>Non-GCB	>Non-GCB	>Non-GCB

CNS, central nervous system; GCB, germinal centre B-cell-like; IV-LBCL-E, intravascular large B-cell lymphoma (Eastern type); IV-LBCL-W, intravascular large B-cell lymphoma (Western type); NR, not reported.

defined. Case 229 presented with HPS, whereas neurological symptoms predominated in case 300. Both cases showed an interstitial and diffuse growth pattern (Figure 3E), and both were CD5-negative. Whereas case 229 presented with a non-GCB phenotype, case 300 showed isolated expression of bcl-6 (without expression of CD10 and IRF4/MUM1), and was therefore classified as being GCB. It also showed diffuse expression of CD30 and a complex karyotype associated with a *bcl-6* rearrangement, whereas *MYC* and *bcl-2* were not rearranged.

Several cases (177, 187, 536, and 255) showed overlapping features that prevented precise classification into one of the above groups. Although most of them were diagnosed as DLBCL NOS according to WHO criteria (often CD5-positive), they were sometimes borderline with IV-LBCL because of the presence of partial intrasinusoidal growth, or might be included in the BSL-LBCL subtype because of interstitial and diffuse growth in the BM associated with spleen and/or liver involvement. The clinical picture was similar, with frequent fever, fatigue, cytopenia, and sometimes suspected or confirmed HPS. Morphologically, all cases showed clear intrasinusoidal growth, accompanied by some degree of extrasinusoidal proliferation, either interstitial (177, 187, and 536) or patchy/nodular (255) (Figure 3F). Three of four cases showed CD5 expression on neoplastic cells, and the same proportion showed a non-GCB phenotype. Complex karyotypes were present in two of the three cases with available information, with a fourth case (255) harbouring a TH configuration (*MYC*, *bcl-2* and *bcl-6* rearrangements), thus fulfilling the WHO criteria for HG-BCL with DH/TH. In this last case, we gave precedence to the genetic features, which are less subjective, although morphological and clinical characteristics overlapping with IV-LBCL were clearly present.

Despite classification difficulties in a given case, it is evident from the workshop series and from literature data that some unifying clinical and pathological

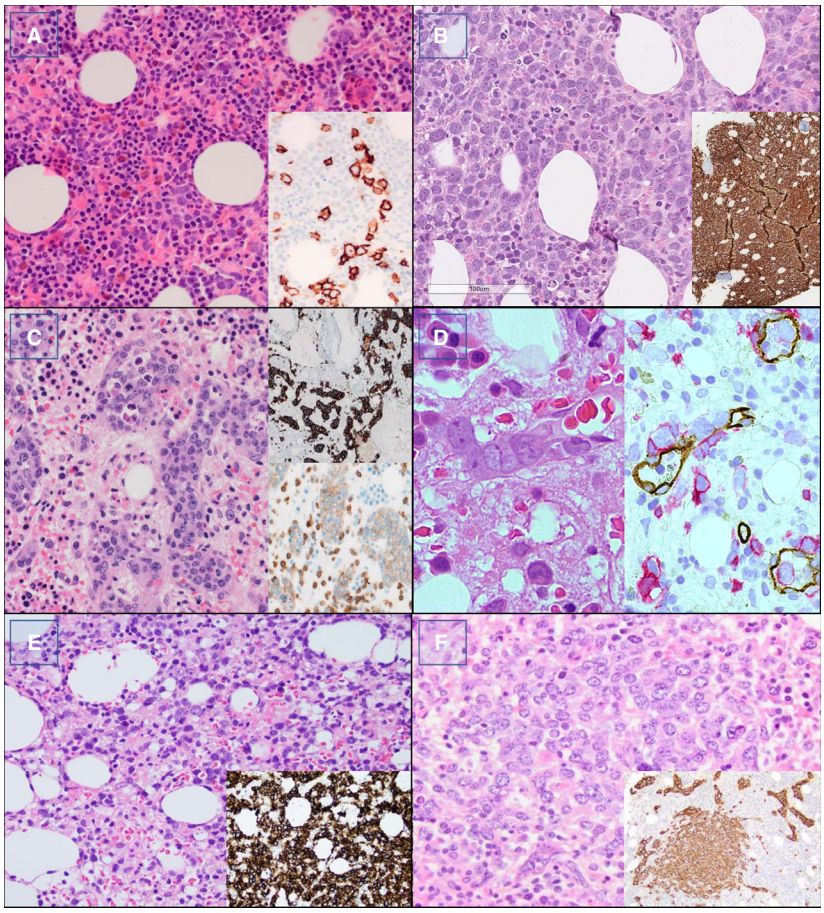

Figure 3. Primary diffuse large B-cell lymphoma of the bone marrow (BM) (PBM-DLBCL), intravascular large B-cell lymphoma (IV-LBCL), BM–spleen–liver large B-cell lymphoma (BSL-LBCL), and unclassifiable cases. **A,B,** Case 481 shows a mainly intravascular pattern of PBM-LBCL in a first biopsy [**A,** haematoxylin and eosin (H&E); and **A,** inset, CD20), but also a diffuse pattern in the second biopsy (**B,** H&E, and **B,** inset, CD20). **C,** Case 215 shows a purely intravascular IV-LBCL with CD5 expression (**C,** upper inset, CD20; and **C,** lower inset, CD5). **D,** A case of IV-LBCL (case 291) with minimal interstitial involvement, as shown in the right panel double staining (CD34 and CD20 staining in brown and red, respectively). **E,** Case 229, an example of BSL-LBCL with interstitial and diffuse involvement of the BM. **F,** Case 255, a triple-hit HG-BCL with nodular and intravascular involvement of the BM.

features characterise these large B-cell lymphomas with a primary BM presentation. From a clinical point of view, most patients present with fever of unknown origin, cytopenia (either unilineage or multilineage), fatigue, a suspected or confirmed HPS, and frequent splenomegaly without lymphadenopathy, occasionally with neurological symptoms, i.e. a clinical constellation usually associated with genuine IV-LBCL. Morphologically, they show a mixture of intravascular/intrasinusoidal growth and 'extravascular' patterns, usually interstitial or patchy, but rarely diffuse. As these patterns might be heterogeneous in spatial distribution, or might change in repeat biopsies (e.g. case 481), one could hypothesise that they constitute a progressive spectrum of BM infiltration, probably reflecting the amount and duration of involvement, and variable cross-talk between the neoplastic cells and their microenvironment, including endothelial and other BM cells. Indeed, most of the described overlap cases could fall within the spectrum of IV-LBCL, as recently described[24]; however, random skin biopsies including subcutaneous fat (recommended to ensure a diagnosis[30]) were not performed in most cases, and there are no clear-cut diagnostic criteria specific for the BM in the WHO classification.[23] Immunophenotypically, the great majority of cases show a non-GCB phenotype, and many of them are also CD5-positive. Although detailed genetic information is lacking for many cases, the reported ones frequently show a complex karyotype and increased frequency of *bcl-6* aberrations (translocations or gains), as well as lesions at the 19q13 locus (212), this latter having been reported in association with BSL-LBCL.[29]

PB-DLBCL

PB-DLBCL is not a lymphoma entity according to the WHO classification of haematological neoplasms, and would be therefore classified as DLBCL NOS.[4] A definition of primary bone lymphoma, however, exists in the WHO classification of tumours of soft tissue and bone.[20] Although, *de facto*, PB-DLBCL also involves the BM, it has been historically separated from PBM-DLBCL on the basis of the absence of lytic bone lesions in the latter. The published criteria for PB-

DLBCL are those of a DLBCL presenting with a single lytic lesion with or without regional lymphadenopathy, or multiple lytic bone lesions without lymphatic or visceral involvement.[25] Morphologically, PB-DLBCL frequently shows a predominance of large cells with multilobulated nuclei.[31] The distinction of PB-DLBCL from other entities seems reasonable in consideration of its good prognosis when it is treated with combination chemoradiotherapy or immunochemotherapy,[32–34] its typical presentation with bone pain (with a low incidence of systemic symptoms), its low International Prognostic Index score, its high prevalence of the GCB phenotype,[32,33] and its GCB signature determined with gene-expression profiling.[33]

Two of the submitted cases presented with lytic bone lesions without lymphadenopathy or other organ involvement, and could therefore be classified as primary bone lymphomas. Case 212 presented with hip pain, weight loss, hypercalcaemia, increased kappa free light chain expression, and multiple, prevalently axial, bone lesions, simulating a plasma cell myeloma. A BM biopsy showed diffuse growth of large cells consistent with DLBCL, positive for CD10, and therefore indicating a GCB phenotype. The karyotype was 47,XY,+X,t(1;3)(q42;q12),add(14)(q32), add(19)(q13.3)[7]/46,XY[43]. Case 214 presented with pain and multiple lytic axial bone lesions. A directed L3 bone biopsy was unsuccessful, whereas standard bilateral BM biopsies showed 50% replacement of the medullary space by an interstitial and diffuse infiltrate of large B cells, compatible with DLBCL. The cells also coexpressed CD30 at consistent levels,

Table 3. Take-home messages

BL
• BL may show minor variations in immunophenotype in otherwise typical cases, such as weak positivity for bcl-2 or MUM1 positivity.
• BL, characterised by BM involvement without lymphadenopathy or extranodal masses, is rare and may occasionally show a more immature phenotype, but is TdT-negative by definition.
• In the absence of a *MYC* translocation, other entities, including Burkitt-like lymphoma with 11q aberration and HG-BCL NOS, need to be considered.
HG-BCL
• Cases of mature aggressive B-cell lymphoma presenting in the BM should be studied for the presence of a *MYC* translocation irrespective of the presence of MYC protein expression.
• The immunophenotypic workup should include TdT and cyclin D1 irrespective of morphology, as cases of B-cell lymphoblastic leukaemia/lymphoma may occasionally show significant pleomorphism, and blastoid mantle cell lymphoma may simulate HG-BCL.
• Aggressive B-cell lymphomas/leukaemias with expression of TdT in the vast majority of neoplastic cells are classified as B-cell lymphoblastic leukaemia/lymphoma irrespective of the presence of a *MYC* translocation with or without *bcl-2* and/or *bcl-6* translocations in the WHO classification. Such cases warrant future studies.
• The diagnosis of HG-BCL NOS requires exclusion of other entities, including blastoid mantle cell lymphoma, B-prolymphocytic leukaemia, and lymphoblastic leukaemia/lymphoma.
• The incidence of BM infiltration in Burkitt-like lymphoma with 11q aberration is unknown. It is of note that the 11q aberration is observed in other entities, including BL with *MYC* rearrangement.
DLBCL
• Most mature large B-cell lymphomas with DLBCL morphology diagnosed in the BM will be assigned to the DLBCL NOS category (often with CD5 expression) or, in the proper clinicoradiological context, to the primary bone DLBCL category.
• Cases without lymphadenopathy might constitute primary DLBCL of the BM or, in the proper morphological and clinical setting, IV-LBCL.
• Large B-cell lymphoma with involvement of the BM, spleen and liver is a novel proposed entity that shows an overlapping morphological and immunophenotypic spectrum with IV-LBCL and primary DLBCL of the BM. Further studies are needed to clarify the exact boundaries between these entities.

BL, Burkitt lymphoma; BM, bone marrow; DLBCL, diffuse large B-cell lymphoma; HG-BCL, high-grade B-cell lymphoma; IV-LBCL, intravascular large B-cell lymphoma.; MUM1, multiple myeloma 1; NOS, not otherwise specified; TdT, terminal deoxynucleotidyl transferase; WHO, World Health Organization.

and showed a non-GCB phenotype. These examples indicate that PB-DLBCLs also show multifocal involvement simulating metastatic disease or plasma cell myeloma.

Differential diagnosis between THRLBCL and NLPHL with a primary BM manifestation

Three cases (429, 499, and 537) with primary BM involvement highlighted the differential diagnostic problem between THRLBCL and NLPHL. This distinction may be already very challenging in some cases presenting in the lymph nodes or spleen, and can be impossible in the BM. Indeed, two of the three cases were eventually classified on the basis of findings described in a lymph node (499) or the spleen (537), whereas case 429 was diagnosed as NLPHL on the basis of the presence of clear-cut programmed cell death-1 (PD-1)-positive T-cell rosettes around large B cells. CD75, a marker that is useful for the distinction of lymphocyte-predominant cells,[35] was also positive in this case; small B cells were present in small numbers. The typical picture of NLPHL in the BM is that of a T-cell and histiocyte-rich pattern, with dispersed large B cells and a variable number of small B cells. Large B cells show a complete B-cell differentiation programme, with expression of CD20, CD79a, OCT2, and BOB1, and they are usually negative (or only weakly positive) for CD30. The only helpful features differentiating THRLBCL and NLPHL in the BM are the abundance of small B cells, the presence of rosette-forming PD-1-positive T cells, and IgD expression (all favouring NLPHL). As many cases might present with borderline features in the BM, it is therefore recommended that a lymph node biopsy should be performed when possible.

Conclusions

Primary manifestations of aggressive B-cell lymphomas may present significant diagnostic problems if no other tissue specimens are available (Table 3). Complete phenotyping, especially in cases with blastoid features, should include TdT, cyclin D1, and SOX11, among other markers, as well as cytogenetics from FISH studies for *MYC*, *bcl-2*, and *bcl-6*. Large B-cell lymphomas with a primary BM presentation include groups of cases with characteristic clinicopathological presentations, but with significant overlap, including IV-LBCL and CD5-positive DLBCL, and BSL-LBCL, requiring further studies for better characterisation.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

A. Zamò reviewed workshop cases, drafted and revised the manuscript, and approved the final version. P. Johnston reviewed workshop cases, drafted and revised the manuscript, and approved the final version. A. D. Attygalle reviewed workshop cases, revised the manuscript, and approved the final version. C. Laurent reviewed workshop cases, revised the manuscript, and approved the final version. D. A. Arber reviewed workshop cases, drafted and revised the manuscript, and approved the final version. F. Fend reviewed workshop cases, drafted and revised the manuscript, and approved the final version.

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