Prognostic factors in diffuse large B-cell lymphoma at diagnosis (excluding the International Prognostic Index)

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Facteurs pronostiques des lymphomes B diffus à grandes cellules au diagnostic en dehors de l'index pronostique international

diffuse large B cell lymphoma, prognosis, cell of origin, MYC/BCL2/BCL6, total metabolic tumour volume, circulating tumour DNA

Abstract

Diffuse large B cell lymphoma (DLBCL) is the most common adult non-Hodgkin's lymphoma, accounting for between 30% and 40% of all lymphomas. The prognosis varies widely, depending on various factors related to the patient (in particular, age and performance status) or to the characteristics of the lymphoma. Initially based on the Ann Arbor staging developed in 1971, the prognostic stratification was later enriched with the International Prognostic Index (IPI) developed in 1993 and revised in 2007 after the introduction of rituximab (R-IPI). Since then, a large number of prognostic factors have been identified, based on morphological (presence or absence of immunoblastic aspect), immunohistochemical (cell of origin, overexpression of MYC and/or BCL2 proteins, expression of TP53 protein, expression of CD30, CD5, PD-1 and/or PD-L1, proliferation index), and molecular (rearrange-

Résumé

Le lymphome B diffus à grandes cellules (DLBCL) est le lymphome non hodgkinien de l'adulte le plus fréquent, représentant 30 à 40 % de l'ensemble des lymphomes. Le pronostic apparait très hétérogène en fonction de différents facteurs liés au patient lui-même (notamment son âge et son statut de performance) et aux caractéristiques de sa maladie. D'abord basée sur le seul stade Ann-Arbor développé en 1971, la stratification pronostique s'est ensuite enrichie avec l'index pronostique international (IPI) élaboré en 1993, et révisé en 2007 après l'introduction du rituximab (R-IPI). Depuis, de très nombreux facteurs pronostiques ont été identifiés, basés sur les caractéristiques morphologiques (aspect immunoblastique ou non), immunohistochimiques (cellule d'origine, sur expression des protéines MYC/BCL2, expression de la protéine TP53, expression du CD30, du CD5, de PD-1 et/ou PD-L1, index de

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ment of *MYC/BCL2/BCL6* genes and mutational profile) characteristics of DLBCLs, and, more recently, information drawn from functional imaging (total metabolic tumour volume) or circulating tumour DNA. All these elements can be taken into consideration when deciding on patient treatment during multidisciplinary consultation meetings, although as of 2021, R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) still remains the reference treatment for DLBCL.

prolifération), et moléculaires (remaniement des genes *MYC/BCL2/BCL6* et profil mutationnel) des DLBCL, et plus récemment à partir de l'imagerie fonctionnelle (volume tumoral métabolique total) ou de l'ADN tumoral circulant. Tous ces éléments peuvent être pris en compte dans la réflexion sur la prise en charge des patients lors des réunions de concertation pluridisciplinaire, bien que le rituximab, cyclophosphamide, doxorubicine, vincristine et prednisone (R-CHOP) reste, en 2021, le traitement de référence des DLBCL.

Diffuse large cell B lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma in adults, accounting for 30%–40% of all lymphomas [1]. It is, by definition, a B lymphoma of diffuse architecture, composed of medium to large cells, i.e., cells with nuclei at least twice the size of the nucleus of a normal lymphocyte [1]. In reality, and in clinical, immunophenotypic and molecular terms, this is a very varied group of lymphomas. As such, the latest 2017 World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues lists a total of more than 15 different subtypes of DLBCL, which can be classified according to, among other things: the primary lymph node or extra-ganglionic site, whether or not they are associated with a virus, an immunosuppressed condition, or specific cytogenetic or molecular abnormalities [1]. If cases do not belong to one of these subtypes, they are referred to as "no other specification" (NOS) DLBCL, which represent a total of 80% of DLBCL cases [1]. Patients' prognoses appear to vary significantly depending on various factors related to either the patients themselves or to the characteristics of their lymphoma. Initially based on the single Ann-Arbor stage developed in 1971, prognostic stratification was later enriched with the International Prognostic Index (IPI) developed in 1993 and revised in 2007, after the introduction of rituximab (R-IPI). Since then, other prognostic factors have been identified, such as MYC/BCL2 protein expression or the presence of MYC/BCL2/ BCL6 gene alterations. All of these elements can be taken into account when deciding upon patient treatment during multidisciplinary consultation meetings, although rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) remain, as of 2021, the reference treatment for DLBCL. The aim of this review is to discuss the role of the main prognostic factors identified to date in DLBCL, other than IPI (*figure 1* and *table 1*). For the sake of clarity, we will limit the discussion to NOS DLBCL and high-grade B-cell lymphoma *(figure 2)* according to the latest WHO classification in 2017. As a reminder, a prognostic factor informs about the probability of an event (such as death) and defines the prognosis of patients in the absence of treatment or regardless of the treatment received. In contrast, a predictive (or theranostic) marker provides information about the likely benefits of a specific treatment programme and thus guides treatment decisions for a given patient.

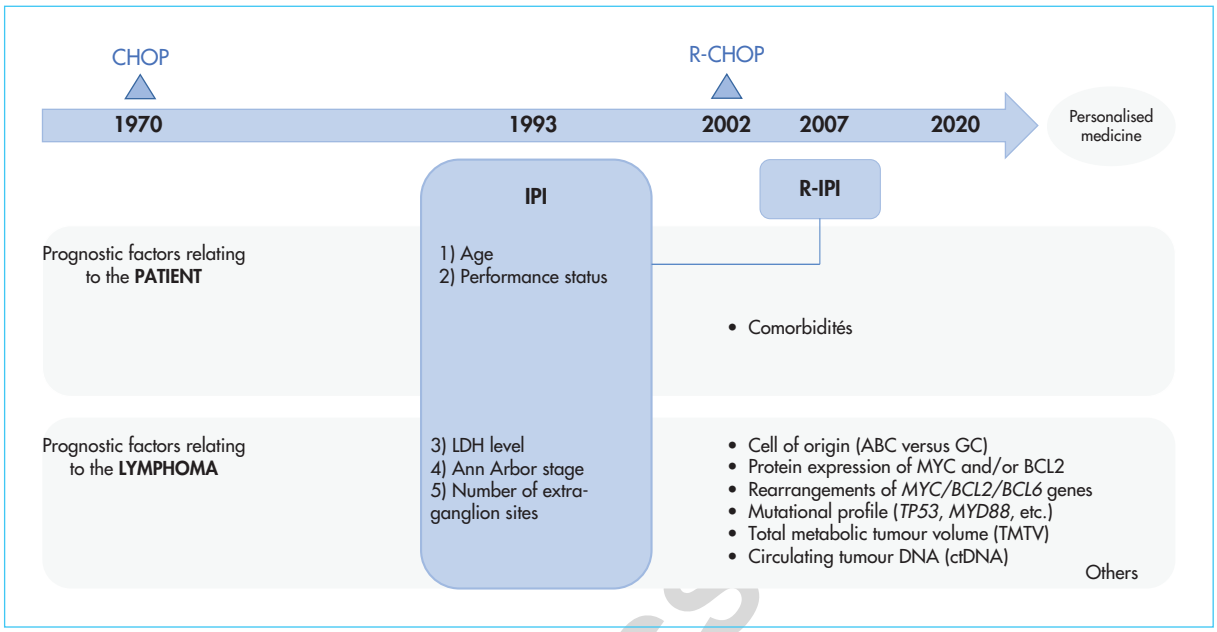
Prognostic factors related to immunomorphological characteristics

Morphological characteristics

The 2017 WHO classification recognises three main morphological aspects: centroblastic (by far the most common), immunoblastic and anaplastic [1].

The immunoblastic variant, defined by the presence of a majority (>90%) of cells with nuclei containing a single large central nucleolus, appears to be associated with reduced overall survival (OS) and progression-free survival (PFS) in some

Figure 1



Main prognostic factors identified in diffuse large cell B lymphoma (DLBCL). ABC: peripheral activated type B DLBCL; GCB: centro-follicular type DLBCL; IPI: International Prognostic Index; R-IPI: Revised IPI; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone.

studies, including patients treated with rituximab [2]. However, this point is controversial according to the authors, especially since interobserver reproducibility was far from excellent (Kappa coefficient = 0.64 in the study by Ott *et al.)* [2]. In practice, this morphological classification is rarely used, but is of interest in highlighting the morphological spectrum of DLBCL.

Immunohistochemical characteristics

Cell of origin (COO)

In the early 2000s, transcriptomic analyses, performed on a DNA chip, made it possible to classify DLBCL into three distinct biological subcategories: germinal centre B-cell-like (GCB), peripheral activated B-cell-like (ABC), and primary mediastinal B-cell lymphoma (PMBL) DLBCL [3]. PMBLs have a molecular signature which is intermediate between classic Hodgkin's lymphoma and DLBCL. GCBs are characterised by a high level of expression of genes usually observed in germinal centre B cells (expression of *CD10*, *BCL6*, *LMO2*, etc.) whereas ABCs have a transcriptomic signature comparable to that of peripheral blood activated B lymphocytes (expression of *MUM1/IRF4*, cyclin D2, *FOXP1*, *BD-2*, etc.). The putative cellular origin of ABC DLBCL is post-centrogerminative. Approximately 10%–15% of NOS DLBCL remain unclassified ("Type 3") with a transcriptomic profile intermediate between that of GCB and ABC DLBCLs. The prognosis of patients appears to be much worse for ABC DLBCL, with a two-year OS rate of 46% versus 78% for GCB DLBCL when rituximab is administered ($p < 0.001$) [4].

Of course, it is not possible to perform a transcriptomic profile for all patients presenting with DLBCL. Different immunohistochemical algorithms have therefore been developed, allowing the GCB versus ABC profile to be deduced, with varying degrees of reliability, according to the immunohistochemical expression of different proteins. Most of these algorithms have a binary result and classify patients by centrogerminative (CG) or non-centrogerminative (NGC) phenotype.

Table 1

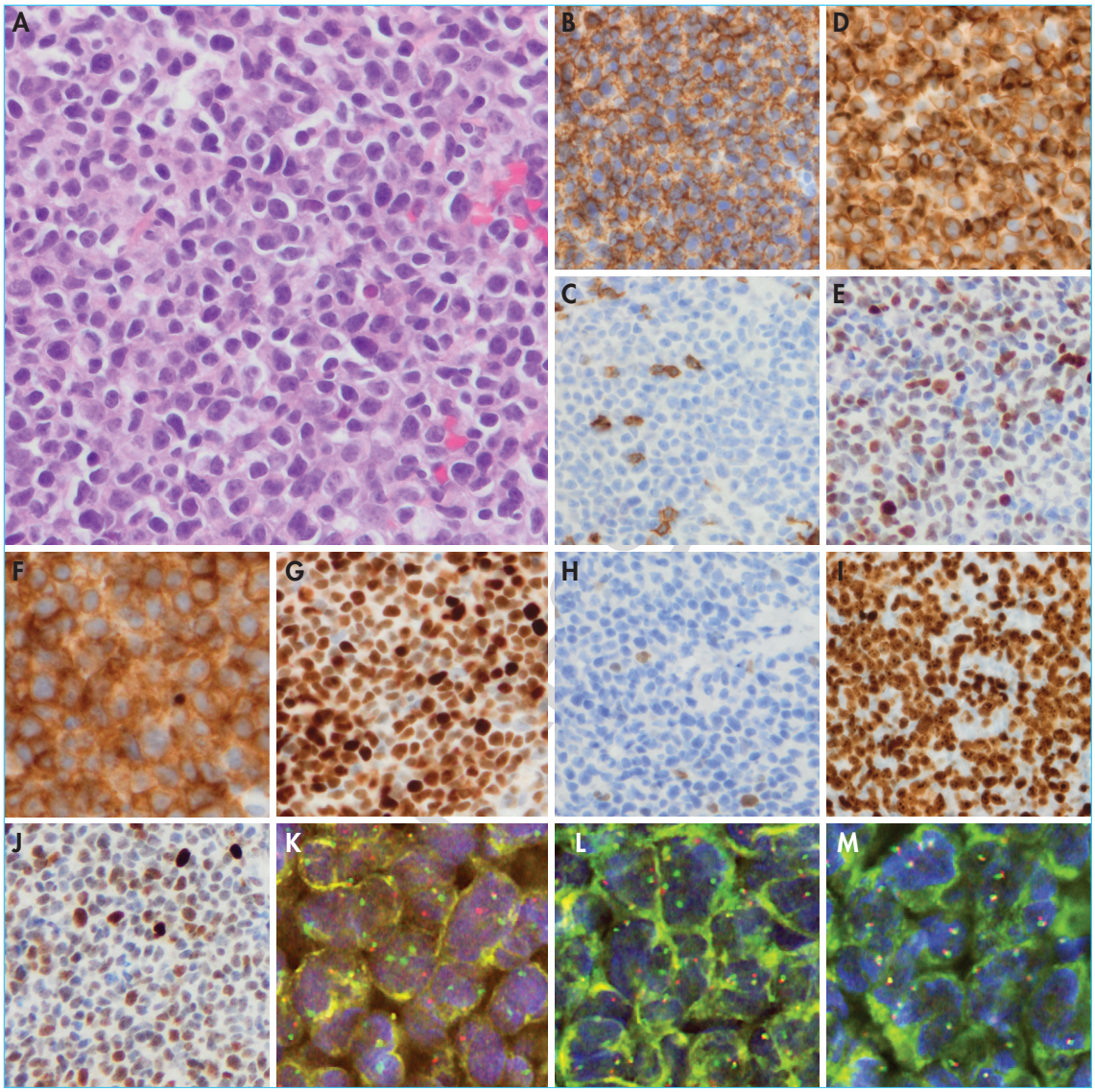
Main prognostic factors identified in diffuse large cell B lymphoma (DLBCL).

	Specialised therapy[a]	Prognostic impact[a]
Morphology		
Centroblastic	No	No
Immunoblastic	No	Debatable
Anaplastic	No	No
Immunophenotype		
GC-like DLBCL (Hans algorithm)	Probably	Debatable
Non-GC-like DLBCL (Hans algorithm)	Probably	Debatable
Co-expression of MYC (>40%) and BCL2 (>50%)	Probably	Yes
Overexpression of TP53 protein (>50%)	No	Yes
CD30 expression	Yes	Yes
CD5 expression	No	Yes
Expression of PD-1 and/or PD-L1	Yes	Debatable
Proliferation index (Ki67)	No	Debatable
Molecular		
GC-like DLBCL (transcriptome)	Probably	Yes
ABC-like DLBCL (transcriptome)	Probably	Yes
Double/triple hitb DLBCL	Probably	Yes
TP53 mutations	No	Yes
Metabolic imaging		
Total metabolic tumour volume (TMTV)	No	Yes
Biology		
Circulating tumour DNA (ctDNA)	No	Yes

ABC: active B-cell-like DLBCL; GC: centrofollicular-like DLBCL.
[a]Compared to "typical" *de novo* DLBCL.
[b]DLBCL with *MYC* and *BCL2* and/or *BCL6* gene rearrangement.

Therefore, the 10%–15% of cases that are molecularly unclassified (type 3, *see above)* are *de facto* associated "by mistake" with one of these GC or NGC categories. The most widely used algorithm is the Hans algorithm, published in 2004, which is based on the use of CD10, BCL6 and MUM1, with a positivity threshold of 30% for each of these three markers. Other algorithms exist such as Visco-Young, Choi, Muris, Nyman and Tally. In practice, the Hans algorithm lacks inter-laboratory and inter-observer reproducibility but remains the only algorithm used in France [5]. Furthermore, while the negative prognosis of ABC DLBCL, as defined by transcriptomic data, is well demonstrated, the negative impact of the NGC

Figure 2



Morphological, immunohistochemical and cytogenetic features of high-grade B-cell lymphoma with rearrangement of the *MYC* and *BCL2* genes ("*double hit*") according to the 2017 WHO classification. Morphologically, the tumour cells are large, arranged in patches with a diffuse architecture (**A**). On immunohistochemistry, the tumour cells are CD20+ (**B**), CD5- (**C**), BL2+ (100%) (**D**), MYC+ (70%) (**E**) and have a centrogerminative phenotype according to the Hans algorithm (CD10+ [**F**], BCL6+ BCL6+ [**G**], and MUM1- [**H**]). The proliferation index (Ki67) is high, above 90% (**I**). There is heterogeneous overexpression of the TP53 protein, of low to moderate intensity, sometimes strong (60%–70%) (**J**). On *in situ* fluorescence hybridisation (FISH), there is a alteration of the *MYC* (**K**) and *BCL2* (**L**) genes, without any associated rearrangement of *BCL6* (**M**).

phenotype based on the use of immunohistochemical algorithms is more debatable [6].

The initially published transcriptomic profiles were based on the use of frozen material. In practice, cryopreserved material is not always available. Therefore, new molecular biological techniques have been developed, allowing the determination of ABC, GCB and "unclassified" profiles from formalin-fixed and

paraffin-embedded material. This is the case, in particular, for NanoString technology (Lymph2Cx assay) and reverse transcriptase multiplex ligation-dependent amplification (RT-MLPA) [7, 8], based on the expression levels of 20 and 14 genes, respectively. The prognostic value of the GCB versus the ABC profile determined by these techniques, both of which are readily available for routine use, has been well demonstrated on OS and PFS [7, 8]. Furthermore, they appear to be more effective in determining the GCB *versus* the ABC profile than immunohistochemical algorithms [7, 8]. Finally, they may lead to the identification of molecularly "unclassified" cases, which is not possible with immunohistochemistry [7, 8].

Overexpression of MYC and/or BCL2 proteins

The prognostic impact of MYC and BCL2 overexpression in DLBCL has been extensively studied. As a reminder, MYC is a transcription factor involved in many cellular functions including apoptosis, proliferation and the cell cycle, while the BCL2 protein promotes cell survival by inhibiting apoptosis. The WHO positivity threshold for defining DLBCL as BCL2+ and/or MYC+ is 50% and 40% of immunostained cells, respectively [1]. MYC overexpression is detected in approximately 30%–40% of all DLBCL cases, more frequently in the ABC subtype than in the GCB subtype [9] (*table 2*). In contrast, overexpression of BCL2 occurs in about 50%–60% of all DLBCL cases, again more frequently in the ABC subtype than in the GCB subtype [9] (*table 2*). With the administration of rituximab, concomitant overexpression of MYC/BCL2 proteins is a factor of poor prognosis for OS and PFS, as demonstrated in numerous studies [1013]. This so-called "double-expressor" phenotype is observed in about 20%–30% of DLBCL NOS and appears more frequently in the ABC subtype than in the GCB subtype [14] (*table 2*). In contrast, the negative impact on survival of isolated MYC or BCL2 overexpression is much more debatable depending on the study [10-13]. The fact that many pathophysiological mechanisms are responsible for the overexpression of MYC/BCL2 proteins, such as translocations, mutations, copy gains, amplifications, and transcriptional deregulation, should be taken into account. The mechanisms involved vary according to the cell of origin; mainly translocation in GCB DLBCL and transcriptional amplification and deregulation in ABC DLBCL [14]. Furthermore, the level of MYC protein expression varies according to the mechanism involved; very intense protein expression in the case of translocation (especially if the MYC partner gene is an immunoglobulin gene and associated with certain mutations) and less so in the case of transcriptional deregulation or amplification [14].

Expression of TP53 protein

The presence of certain mutations in the tumour suppressor gene TP53 (about 20% of DLBCL cases) is associated with a significant reduction in OS and PFS, confirmed in multivariate analysis [15, 16]. This reduction in survival is still valid with rituximab, and applies to both GC and NGC DLBCL [15, 16]. In contrast, deletion in *TP53* and loss of heterozygosity are not associated with decreased survival [16]. In practice, it is not possible to systematically sequence the *TP53* gene in the work-up of any DLBCL. In contrast, accumulation of the TP53 protein, as a result of a mutation in its gene, can be easily detected using an immunohistochemical technique. Indeed, the majority of TP53 mutations are missense mutations resulting in the synthesis of a stable but inactive protein that accumulates in the nucleus of cells [17]. For example, it has been shown in several studies that overexpression of TP53 in DLBCL is associated with reduced survival [17, 18]. The most commonly used threshold is positivity in 50% of immunohistochemically stained cells.

Table 2

MYC, _BCL2_ and _BCL6_ in diffuse large cell B lymphoma (DLBCL).

	DLBCL	GCB-like	ABC-like
Protein expression (immunohistochemistry)			
BCL2 expression (>50%)[a]	66%	58%	85%
MYC expression (>40%)[a]	41%	32%	61%
MYC/*BCL2* double expressor[a]	31%	20%	53%
Chromosomal changes			
Translocation of *BCL2*[a]	27%	46%	6%
Translocation of *BCL6*[a]	22%	15%	29%
Translocation of *MYC*[b]	11.6%	16.6%	6.3%
MYC-SH	35.5%	28.4%	60%
MYC-DH BCL2	39.0%	48.2%	7.5%
MYC-DH BCL6	13.6%	8.8%	30.0%
MYC-TH	11.9%	14.6%	2.5%
MYC partner gene[b]			
MYC/*IG*	56%	54. 8%	59.5%
MYC/non-IG	44%	45.2%	40.5%
MYC copy gains (3–4 copies)[c,d]	19%–38%	ND	ND
MYC amplification (> 4 copies)[d]	2%	ND	ND
Somatic mutations			
MYC mutations[e]	32%	ND	ND

ABC: activated B-cell-like DLBCL; DH: *double* hit i.e. DLBCL with MYC and BCL2 or BCL6 GC, centrofollicular type DLBCL *IG* immunoglobulin gene; ND: data not available; SH: *single hit* i.e. DLBCL with isolated rearrangement of the MYC gene; TH: *triple hit* i.e. DLBCL with rearrangement of all three genes, MYC, BCL2 and BCL6.

[a] Study by Scott *et al.* (2015) [9]. Results are based on 335 biopsies of which 108 (32%) were ABC, 189 (56%) were GCB and 38 (12%) were molecularly "unclassified" biopsies by Nanostring (Lymph2Cx assay).

[b] Study by Rosenwald *et al.* (2019) [28]. The results are based on 1,919 biopsies tested by FISH for MYC/BCL2/BCL6 genes with cell of origin determined by Hans immunohistochemical algorithm and/or molecular analysis. A total of 996 (52%) biopsies were GCB and 923 (49%) were non-GCB.

[c] Study by Stasik et al (2010) [40]. The results are based on in situ hybridisation analysis of 52 cases of DLBCL.

[d] Study by Valera *et al.* (2013) [12]. The results are based on in situ hybridisation analysis of 219 cases of DLBCL.

[e] Study by Pasqualucci *et al.* (2001) [41]. The results are based on the sequencing analysis of 39 cases of DLBCL.

CD30 expression

CD30 expression is seen in approximately 10%–20% of all DLBCLs, with equivalent proportions in GCB and ABC DLBCL [1]. Although the data are contradictory between studies, it appears that DLBCL NOS CD30+ is associated with a better prognosis than those that are CD30- [19]. Furthermore, the study of CD30 immunohistochemical expression is now becoming essential with the advent of brentuximab vedotin (an anti-CD30 monoclonal therapeutic antibody).

CD5 expression

CD5+ DLBCLs account for approximately 5%–10% of all DLBCLs [1]. They usually occur de novo or more rarely as part of a transformation from chronic lymphocytic leukaemia/lymphoma. These lymphomas usually involve older patients, with a performance score greater than 1, increased LDH, B symptoms, Stage III/IV, bone marrow invasion and a higher IPI score [20, 21]. In multivariate analysis, CD5+ status itself appears to be associated with reduced OS and PFS [21]. It should be recalled that in the case of any CD5+ lymphoma, a cyclin D1 ± SOX11 test should be performed in order not to overlook possible mantle cell lymphoma (for aggressive blastoid or pleomorphic variants).

Expression of PD-1 and/or PD-L1

The expression of PD-1/PD-L1 by tumour cells and/or immune microenvironment cells (IMCs) is a hot topic, since the dawn of monoclonal antibodies targeting these two molecules in certain haemopathies. PD-L1 is expressed in approximately 20%–30% of DLBCL cases, although there is wide variation between studies depending on the threshold of positivity used and the cell compartment analysed [22]. This expression of PD-L1 seems to be more common in NGC DLBCL than in GC [22]. In contrast, PD-1 is barely expressed in tumour cells but relatively frequently, up to 60% of the time, in TILs [22]. Prognostically, it appears that a high level of PD1+ TILs is associated with improved survival, whereas a high level of PD-L1+ tumour cells is associated with a poor prognosis [22]. In practice, there is currently no routine assessment of PD-1/PD-L1 expression in DLBCL.

Proliferation index (Ki67)

Some studies have suggested that a high proliferation index, as assessed by the Ki67 antibody, is a poor prognostic factor [23, 24]. In practice, this point is highly debatable. It should also be noted that no threshold is defined in the latest 2017 WHO classification to define a DLBCL as “highly proliferative” [1].

Prognostic factors related to molecular characteristics

Rearrangement of MYC/BCL2/BCL6 genes

Chromosomal translocations involving the *MYC/8q24* gene were first described in Burkitt's lymphoma as a t(8;14) translocation between the *MYC* gene and the immunoglobulin heavy chain gene (*IGH*). More rarely, other partners have been described such as the k light chain gene *(IGK)* and the l light chain gene (*IGL*). In all cases, the *MYC* partner is, in Burkitt's lymphoma, an immunoglobulin gene *(IGH, IGK* or *IGL)* and this translocation may be identified in a simple karyotype.

DLBCL can also be affected by these translocations, but these are part of a complex karyotype. However, unlike Burkitt's lymphoma, the *MYC* partner may be an immunoglobulin gene or another gene (in slightly fewer than half of the cases) such as *BCL6, BCL11A, PAX5, IKAROS, BTG1, etc.* [25] *(table 2*). In addition, other molecular abnormalities involving *MYC* can be observed such as copy gain (three or

four copies), amplification (more than four copies) or mutations in 21–38%, 2% and 32% of cases, respectively [25] (*table 2*).

In practice, *MYC* translocation is observed in 5–17% of DLBCL cases, more frequently in the GC group than in NGC [26] (*table 2*). In about one third of cases, it is an isolated rearrangement of *MYC*, i.e., without associated alteration of the *BCL2* and/or *BCL6* genes (single hit) (*table 2*). In the remaining two-thirds of cases, there is an associated rearrangement of *BCL2/18q21* or *BCL6/3q27* (double hit) or both genes (triple hit) [26] (*table 2*). Since the new 2017 WHO classification, double/triple hit DLBCLs are separately classified as "high-grade B lymphoma with *MYC* and *BCL2* and/or *BCL6*". It should be noted that patients with an isolated rearrangement of *MYC* or presenting with a rearrangement of *BCL2* and/or *BCL6* without associated rearrangement of *MYC*, rearrangement of *MYC* associated with a rearrangement not involving *BCL2* or *BCL6* (e.g. *CCND1*), or amplification without translocation of the *MYC* gene, are always classified as DLBCL NOS or high-grade B-cell lymphoma type NOS according to the morphology of the tumour cells [1]. It should also be noted that the *MYC* partner gene is not included in the double/triple hit category.

These molecular abnormalities have an impact on prognosis, which is why it is important to look for them. Isolated *MYC* rearrangement appears in itself to be a factor for poor prognosis in terms of OS and PFS, if and only if the partner gene is an immunoglobulin, although this is somewhat discordant between studies [27, 28]. Double and triple hit rearrangements are, in all studies, a factor for poor prognosis in terms of OS and PFS, if and only if the partner gene of *MYC* is also an immunoglobulin [28]. There is no prognostic difference between double and triple hit [28]. Similarly, there is no difference between double hit with a rearrangement of *BCL2* and double hit with a rearrangement of *BCL6* [28]. Finally, interestingly, it appears that "double-expressor" DLBCLs have an intermediate prognosis between that of DLBCL NOS and double/triple hit DLBCLs [11].

On a day-to-day basis, the main difficulty is knowing when to carry out an *in situ* hybridisation (FISH) study of the *MYC/BCL2/BCL6* genes. This question is all the more fundamental as FISH is a relatively expensive and time-consuming technique. There is currently no "official" recommendation on this issue, but various studies have evaluated the sensitivity and specificity of different pre-screening strategies based on immunophenotypic characteristics [26]. Unfortunately, there is no absolute correlation between the level of MYC/BCL2 protein expression and the presence of a remodelling of these two genes. In theory, the only way to detect all *double/triple hit* DLBCLs is to systematically perform FISH on all patients, either by analysing all three MYC/BCL2/BCL6 genes or only MYC, and then complementing with BCL2/ BCL6 in case of a rearrangement of MYC [29]. By restricting FISH to DLBCL of centrogerminative origin according to the Hans algorithm, the sensitivity and specificity for detecting *double/triple hit* DLBCL are 92% and 52%, respectively [26]. Similarly, limiting FISH to DLBCLs of centrogerminative origin according to the Hans algorithm and expression of MYC protein on immunohistochemistry (at the 40% threshold), sensitivity and specificity are 74% and 85%, respectively [26]. In conclusion, it is up to each centre to determine which strategy to adopt, depending, in particular, on the potential therapeutic impact.

Mutation profile

The mutational profile of DLBCL is now well known, particularly as the result of the development of next-generation sequencing techniques (NGS) [30-32]. Recurrent mutations detected by NGS are now part of the molecular identity map of DLBCL, as are recurrent translocations or the transcriptomic profiles described above. Overall, the oncogenic pathways involved appear to be very different depending on the cell of origin. Schematically, ABC DLBCL is characterised by chronic BCR

activation related to mutations in the CD79A/CD79B subunits of the receptor, which are observed in approximately 20–25% of cases [1]. This is associated with constitutional activation of the nuclear factor kB (NF-kB) pathway, resulting from multiple mutations in positive regulators *(*CARD11, TRAF2, TRAF5, MAP3K7, and TNFRSF11A*)* or negative (*A20*) regulators of this pathway. In particular, the CARD11 gene is found to be mutated in approximately 10–15% of ABC DLBCLs. Finally, a mutation in the MYD88 gene is observed in about 35% of ABC DLBCLs, promoting the formation of interleukin-1 receptor-associated kinase-4 (IRAK-4) protein complexes and IRAK-1, activation of the NF-kB pathway, activation of signal transducer and activator of transcription 3 (STAT3) by Janus kinases (JAK) and secretion of interleukin 6 (IL-6), IL-10 and interferon B [1]. Of note, breast, skin, gastric and testicular primary DLBCLs, as well as those affecting the central nervous system, are more frequently of the ABC subtype and associated with mutations in MYD88. In contrast, GCB DLBCLs are characterised by mutations in EZH2, regulating histone methylation, in 20–25% of cases, and GNA13 in about 25% of cases [1]. The identification of these distinct molecular pathways has enabled the development of targeted therapies. This is the case for ibrutinib (a Bruton's tyrosine kinase [BTK] inhibitor) and lenalidomide (of the immunomodulatory drug [IMiD] class), both of which are effective primarily in ABC DLBCL [33].

Some very recent studies aim to create molecular subcategories based on the detailed mutational profile. For example, Lacy *et al.* [30] identified six groups of DLBCLs, named according to their main molecular abnormality: MYD88, NOTCH2, TET2/SGK1, BCL2, SOCS1/SGK1 and not elsewhere classified (NEC). Some of these subcategories appear to be fairly robust and have been identified in various independent studies (in particular the MYD88, BCL2, and NOTCH2 clusters) [30]. The SOCS1/SGK1, BCL2 and TET2/SGK1 categories are mainly concerned with GCB DLBCLs, whereas the category MYD88 is more concerned with ABC DLBCLs [30]. The NEC and NOTCH2 categories are composite, combining GCB, ABC and unclassified DLBCLs [30]. Interestingly, the prognosis of patients seems to differ according to molecular subcategories [30].

In conclusion, in the future, it would be interesting to identify certain mutations of diagnostic, prognostic and theranostic value by targeted sequencing of a restricted panel of genes (about 20–30 genes). These genomic results would then be discussed at a molecular multidisciplinary consultation meeting in order to adapt patients' treatment.

Total metabolic volume assessed by functional imaging

Imaging techniques play a central role in identifying DLBCL by accurately assessing the different anatomical compartments affected. Since the “simple” scanner, other imaging techniques have been developed. This is particularly the case for positron emission tomography (PET), based on the cellular avidity of glucose (18-fluorodeoxyglucose [18-FDG]). This technique appears to be very sensitive for the detection of various lymphomatous areas, particularly extraganglionic and especially medullary, where PET appears to be more sensitive compared to osteomedullary biopsy [34]. In practice, the radiologist identifies all the hypermetabolic lymph node and extra-ganglion areas and specifies the standardised uptake value (SUV) which reflects the metabolic activity of tumour cells. Since 2007, PET has been considered the main imaging technique for staging aggressive lymphomas, except for neuromeningeal involvement, for which MRI is preferred. The better assessment of extra-ganglionic tumour sites has improved the prognostic value of IPI and R-IPI and has, thus, ultimately improved the management of patients [35].

The initial total metabolic tumour volume (TMTV), i.e., measured before any treatment, has been shown to be of prognostic value in several retrospective series of DLBCL patients, but also for other lymphomas such as Hodgkin's lymphoma, follicular lymphoma and primary mediastinal B lymphoma (PMBL). This parameter combines tumour size (volume) and metabolic activity measured by 18-FDG avidity. In the study by Sasanelli *et al.* [36] published in 2014, patients with TMTV >550 cm^3 had a significantly worse prognosis, with a three-year OS rate of 60%, compared to 87% for those with TMTV <550 cm^3 (p = 0,0003). In the same study, TMTV was an independent prognostic factor for OS (p = 0.002) after adjustment for IPI and bulky tumour mass. To go further, Cottereau *et al.* [37] showed in 2016 that TMTV allowed for better stratification of the prognosis of DLBCL GC *versus* ABC; GC patients with TMTV <300 cm^3 had a five-year OS rate of 87%, compared with 60% for those with TMVT >300 cm^3. Similarly, the survival rate was 60%, compared with 23% for ABC patients with TMTV <300 cm^3 *versus* TMVT >300 cm^3 [37]. Similar results have been obtained by stratifying patients according to the immuohistochemical expression of MYC and BCL2 [38]. Finally, in 2020, Vercellino *et al.* [38] analysed data from the REMARC trial, comparing the efficacy of maintenance treatment with lenalidomide or placebo in patients aged 60–80 years with DLBCL with complete or partial response to R-CHOP. The authors showed that TMTV >220 cm^3 was associated with a significant reduction in OS and PFS, regardless of maintenance treatment (lenalidomide or placebo). They also developed a model based on TMTV and ECOG (Eastern Cooperative Oncology Group) performance status to stratify patients into three different prognostic groups.

Circulating tumour DNA

Circulating tumour DNA (ctDNA) is increasingly used in oncology, particularly for prognostic or theranostic purposes, to predict response to certain targeted therapies. Regarding DLBCL, Kurtz *et al.* [39] observed in 2018 that pre-treatment ctDNA was detectable in 98% of 217 patients tested using Cancer Personalised Profiling with Deep Sequencing (CAPP-Seq). The ctDNA level was also correlated with IPI and TMTV, suggesting that this biological parameter is a good reflection of the degree of tumour invasion. The authors have shown that a high level of ctDNA pre-treatment is a poor prognostic factor in terms of OS and PFS based on univariate analysis [39]. Based on multivariate analysis, after adjusting for IPI, cell of origin and TMTV, pre-treatment high ctDNA levels remained associated with reduced PFS but not OS [39]. The authors then looked at the kinetics of the decrease in ctDNA between the level measured before treatment and that on the first day of the second cycle of chemotherapy (early molecular response for a 2-log decrease) or on the first day of the third cycle of chemotherapy (major molecular response for a 2.5-log decrease) [39]. Interestingly, an early molecular response was associated with improved PFS and OS, both for first-line and rescue treatment. Furthermore, a major molecular response was associated with improved PFS and OS with first-line therapy. In total, ctDNA, which is very easily assessed routinely by non-invasive sampling (simple blood test), should, in the coming years, constitute a new interesting prognostic biomarker in the management of DLBCL patients.

Conclusion

The prognosis of DLBCL appears to vary significantly depending on many clinical, biological and radiological criteria. With a better understanding of the prognosis of patients, it will be possible to tailor treatment to individual cases. Furthermore, the identification of distinct oncogenic pathways in DLBCL will allow therapies to be proposed that target the pathophysiology of lymphoma.]

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