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Original contribution

Clinicopathological, cytogenetic, and molecular profiles of primary cutaneous diffuse large B-cell lymphomas☆,☆☆

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Summary We analyzed the clinicopathological, cytogenetic, and molecular features of 18 primary cutaneous diffuse large B-cell lymphomas (PCDLBCLs) and 15 DLBCLs secondarily localized to the skin (SCDLBCLs), highlighting biological similarities and differences between the 2 groups.

PCDLBCLs were subclassified after histopathological review as PCDLBCL-leg type (PCDLBCL-LT, 10 cases) and the PCDLBCL-not otherwise specified (PCDLBCL-NOS, 8 cases). Immunohistochemistry for Hans' algorithm markers, BCL2, and MYC was performed. The molecular study included the determination of the cell of origin (COO) by Lymph2Cx assay on NanoString platform, FISH analysis of *IgH*, *BCL2, BCL6,* and *MYC* genes, as well as the mutation analysis of *MYD88* gene.

In immunohistochemistry analysis, BCL2 and MYC hyperexpression was more frequent in LT than in NOS cases and, according to Hans' algorithm, PCDLBCL-LTs were mostly of the non-GC type (8/10), whereas in PCDLBCL-NOS, the GC type prevailed (6/8). The determination of COO using Lymph2Cx supported and further confirmed these results. In FISH analysis, all but one LT cases versus 5 of 8 PCDLBCL-NOS showed at least one gene rearrangement among *IgH, BCL2, MYC,* or *BCL6*. In addition, *MYD88* mutations were more frequently present in LT than in NOS subtypes. Interestingly, *MYD88*-mutated patients were older, with a non-GC phenotype and had worse OS, compared to *MYD88* WT cases. Overall, SCDLBCL did not show, at the genetic and expression level, different profiles than PCDLBCL, even if they bear a significantly worse prognosis. At survival analysis, the most important prognostic factors in patients with PCDLBCL were age and *MYD88* mutation, whereas relapse and high Ki-67 expression were relevant in patients with SCDLBCL.

Our study comprehensively analyzed the clinicopathological and molecular features of PCDLBCL-LT, PCDLBCL-NOS, and SCDLBCL, underlining the differences among them and the importance of properly identifying these entities at the time of diagnosis.

1. Introduction

In the last two decades, the availability of high-throughput molecular technologies has expanded our knowledge on the pathogenesis of diffuse large B-cell lymphomas (DLBCLs). The seminal study by Alizadeh and coworkers established the distinction between a germinal center B-cell (GC) subtype and an activated B-cell (ABC) subtype, according to the putative cell of origin (COO), by using gene expression profiling (GEP) of fresh frozen tissue samples [1]. The most common surrogate of GEP in paraffin-embedded material used in daily diagnostic practice is the immunohistochemical algorithm known as Hans' algorithm, which classifies DLBCL in GC and non-GC subtypes based on the expression of CD10, BCL6, and MUM1 [2]. The 2017 World Health Organization (WHO) recommended the application of Hans' algorithm as having predictive and prognostic value [3]. However, the availability of new technologies allowing GEP on paraffin-embedded samples, such as Lymph2Cx assay, has provided a more accurate and prognostically more powerful tool for subclassifying DLBCL according to the COO [4]. The concordance between Lymph2Cx assay and Hans' algorithm is around 70−80% [4−6]. In addition, alterations of lymphoma-related genes, such as rearrangements of *BCL2*, *BCL6,* and *MYC*, as well as mutations of *MYD88* have been reported to have an impact on patients' outcome and response to therapy [7]. More recently, additional genetically determined DLBCL subtypes have emerged, ie, the MCD type, characterized by coexistent *MYD88*L265P and *CD79B* mutations; the BN2 type, characterized by *BCL6* fusions and *NOTCH2* mutations; the N1 type with *NOTCH2* mutations; and the EZB type with *EZH2* mutations and *BCL2* translocations [8].

Primary cutaneous diffuse large B-cell lymphomas (PCDLBCLs) are DLBCLs with exclusive localization to the skin at diagnosis [9]. According to the updated WHO/EORTC classification [10], most PCDLBCLs are included in the leg-type category (PCDLBCL-LT), which consists of a diffuse proliferation of immunoblasts and centroblasts, typically occurring in elderly women, mainly with lesions located in the lower limbs, and with a 5-year survival of 50%, when treated by immunochemotherapy (R-CHOP regimens) [10]. PCDLBCL-LT is similar to other DLBCLs of non-GC/ABC type, but some authors suggest much greater heterogeneity in terms of COO [4,11,12]. Mutational and cytogenetic studies have highlighted that many PCDLBCL-LT do have a mutational/cytogenetic profile similar to other DLBCLs of MCD type, a category that also includes many of the lymphomas from other extranodal primary sites, including the central nervous system and the testis. In practice, PCDLBCL-LT cases must be

distinguished from the group of primary cutaneous follicular cell lymphomas—large cell (PCFCL-LC) exhibiting a predominance of large cells, because the latter shows an excellent outcome (5-year overall survival of 95%) and need a different therapeutic approach based on radiotherapy rather than immunochemotherapy [13,14]. *MYD88* mutation and DH/TH status seem to have a negative impact on prognosis also in PCDLBCL [15–17]. In recent years, different authors have suggested the existence of an additional category of PCDLBCL, characterized by peculiar clinicopathological features [18]. This new entity has been termed firstly *Other* and then *Not-Otherwise Specified* (NOS) and seems to preferentially affect young male patients, with lesions localized in the head and the trunk, displaying intermediate prognosis between PCFCL-LC and PCDLBCL-LT [19,20]. This entity exhibits a diffuse proliferation of centroblasts with a minority of centrocytoid cells counting less than 10% and/or with an inflammatory background. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas gives specific indication to make this diagnosis exclusively in those rare cases that cannot be classified as PCDLBCL-LT or PCFCL-LC [10]. Conversely, the results of a multicenter Italian study indicate that the percentage of PCDLBCL-NOS (ie, not classifiable as PCFCL-LC or PDLBCL-LT based on the clinicopathologic features) cannot be accounted as definitely rare [20].

We aimed to contribute to this discussion by collecting and analyzing the clinical, morphological, phenotypical, genetic, and molecular features of a series of PCDLBCL and, for comparison, of DLBCL secondarily localized to the skin (SCDLBCL), which were diagnosed and treated in 4 Italian academic centers. Herein, we highlight biological similarities and differences between primary and secondary cases, particularly focusing on the genetic and molecular features.

2. Patients and methods

2.1. Patients

We retrospectively analyzed clinical data and skin biopsies of 33 patients diagnosed with DLBCL localized to the skin between 2001 and 2019 in 4 Italian University Hospitals (Ancona, Modena, Pisa, and Varese). Thirteen patients were studied both at diagnosis and at relapse. Cases included in this study were not previously included in other series. We adopted the following inclusion criteria: (1) cases diagnosed as PCDLBCL and SCDLBCL; (2) availability of histopathological samples; and (3) availability of clinical and follow-up data.

Patients were diagnosed as having PCDLBCL when they had no previous history of systemic B-cell lymphoma and the cutaneous lesion was the only localization of disease, as confirmed by clinical and radiological examination. All the other patients were considered as having SCDLBCL.

The study was approved by local institutional review boards and conducted as per the revised Helsinki Declaration.

2.2. Histopathological review

During a session at a multihead microscope held in Modena in June 2021, the 4 pathologists involved in the study (A.M., V.D., G.G., and S.U.) reviewed all the available slides stained with hematoxylin-eosin and/or Giemsa stain, as well as the immunohistochemical staining. The minimum immunohistochemical panel included CD20, CD3, CD10, BCL6, MUM1/IRF4, BCL2, MYC, and Ki-67. When one or more immunostains were lacking, they were performed following local protocols on microtomic sections obtained from representative paraffin blocks (Table S1 in Supplementary Material for antibodies and automated platforms). The immunohistochemical markers of the Hans' algorithm, ie, CD10, BCL6, and MUM1/IRF4, were scored as positive when their expression was observed in $\geq$30% of the tumor cells. According to Hans' algorithm, cases were classified as *GC* and *non-GC* [2]. MYC and BCL2 immunostains were scored positive if $\geq$40% and $\geq$50%, respectively, of neoplastic cells were immunoreactive. Specimens characterized by the co-expression of MYC and BCL2 were defined as double expressors (DEs). The proliferation index was evaluated as the percentage of tumor cells with nuclear expression of Ki-67. Details on antibodies and platforms used in each laboratory are reported in Table S1.

For this study, we have not included cases with typical features of PCFCL-LC, showing a significant amount of medium centrocytes with a typical germinal center phenotype. Based on the histopathological and immunohistochemical features, integrated with clinical data, the PCDLBCL cases were subdivided into PCDLBCL-LT and PCDLBCL-NOS, according to the recognized criteria [10,18–20]. After having excluded PCDLBCL-LT and PCFCL-LC with diffuse growth rich in large or spindled cells, cases composed of more than 90% of large blasts, without follicular dendritic cell meshwork, and a mild T-cell infiltrate, were included in the PCDLBC-NOS group.

2.3. FISH analysis

Interphase FISH analysis was performed and interpreted at the Laboratory of Cytogenetics of Varese University Hospital by 2 expert operators (S.F. and M.G.T.). Three-micrometer-thick sections obtained from a representative paraffin block of each case were used, and 2 hematoxylin and eosin–stained slides, obtained before and after the section used for FISH analysis, were observed to confirm the presence of an adequate number of evaluable lymphomatous cells for genetic analysis. FISH experiments were carried out as described elsewhere using split-signal probes for *BCL2*, *BCL6*, *MYC,* and *IGH* genes, provided by

ZytoVision [21]. The analysis was performed using BioView (Abbott) automated system. The presence of rearranged alleles was defined when the distance between red and green spots exceeded 3 times the fusion signal diameter. The 2 operators analyzed each FISH experiment blindly. In each case, more than 100 nuclei on paraffin-embedded sections were examined from at least 5 to 8 areas selected for well-preserved cellular and nuclear morphology to ensure representative samples and to avoid nuclear truncation. Only experiments with 100% hybridization efficiency were considered. The threshold values for the presence of both specific chromosome rearrangements and trisomy or polysomy were evaluated for each probe on 10 formalin-fixed paraffin-embedded (FFPE) sections of reactive hyperplastic lymph nodes showing normal karyotypes by conventional cytogenetics. The cut-off points were calculated as the mean value plus 3 standard deviations of nuclei showing split signals and 3, 4, and more than 4 spots; the standard deviation was calculated assuming a binomial distribution of the spots. The cut-off value was evaluated for each probe both for rearrangement (5% of cells) and polysomy (18% of cells).

2.4. *MYD88* mutation by next-generation sequencing

Briefly, analysis of *MYD88* mutations was performed by next-generation sequencing (NGS) with an amplicon-based strategy, covering exon 5. Two 10-μm FFPE sections of each specimen were collected and underwent both DNA and RNA extraction using the All Prep DNA/RNA FFPE Kit. Specific primers were designed with the Primer3 program and modified according to the Illumina protocol. Amplicon libraries were generated using a modified Illumina protocol starting from 40 ng of DNA ($\sim$6000 diploid genomes), a quantity capable of successfully detecting mutations below the 1% variant allele fraction (VAF) in the context of our procedures. Multiplex polymerase chain reaction products were purposely designed to avoid the generation of small amplicon products and generated using Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific). Fragments were subsequently tagged with specific indexes according to the DNA Library Preparation Kit (Illumina; San Diego, CA), and purified libraries were normalized, pooled, and paired-end sequenced in a MiSeq instrument (Illumina) with a 2 $\times$ 250 run. Sequencing data generated from NGS experiments were analyzed to identify single nucleotide variants. Alignment to the reference genome (hg19) and variant calling for all samples were performed with MiSeq Reporter software (Illumina), and variants were reviewed through the Integrative Genomics Viewer (Broad Institute, Cambridge, MA). Somatic variants included for analysis passed the MiSeq Reporter quality filter and met laboratory-defined thresholds of $\geq$1000$\times$ coverage and $>$5% VAF.

2.5. COO classification by Lymph2Cx assay on NanoString platform

RNA obtained from FFPE sections was used to perform COO classification through the Lymph2Cx assay on the NanoString platform (NanoString; Seattle, WA). NanoString technology is characterized by a dual-probe system, which contains a combination of target-specific capture probes and reporter probes that allows performing unique counting of RNA target molecules from high-degraded starting material, such as FFPE samples. Each target-specific barcode attached to reporter probes refers to a single transcript of interest, which can be individually counted without amplification or reverse transcription. Based on a 20-gene signature, the Lymph2Cx panel subdivides DLBCLs into ABC, GC, and unclassified subsets.

2.6. Statistical analysis

Patient features are summarized by frequency (percentage) for categorical variables and median (range) values for continuous variables. Statistical analysis was performed using JMP 14.0 software (SAS Institute; Cary, NC), and significance was defined as $P < .05$. The association between clinical presentation and molecular/pathological characteristics was assessed by the χ^2 or Fisher's exact test for nominal data and the Wilcoxon rank sum test for continuous variables. Overall survival (OS) and progression-free survival (PFS) were defined as the time from diagnosis until death from any cause or last follow-up and the time elapsed between treatment initiation and disease relapse/progression or death from any causes, respectively. Survival data were evaluated by the Kaplan-Meier method, with differences between groups compared by the log-rank test.

3. Results

3.1. Clinical features

Among 33 DLBCL patients with cutaneous involvement, we had 18 cases of PCDLBCL and 15 of SCDLBCL. Patients with PCDLBCL were more commonly female (61.1%), with a median age at diagnosis of 75.5 years. The majority presented with lesions located in the legs (66.7%), nearly all without B symptoms. The majority of these patients received local and systemic combined therapy (64.7% of cases). After first-line treatment, 94.1% of patients achieved a complete response (CR), and recurrences occurred in 33.3% of cases, with a median time to relapse of 28.1 months (range, 7.6-73.2 months). Conversely, patients with SCDLBCL were male in 66.7% of cases, the most frequent localization was the trunk, and almost half of the patients experienced B symptoms. All patients received systemic or combined therapies; 58.3% achieved a CR, and 46.7% of them relapsed, with a median time of 15.5 months (range, 1.9-51.9 months). Details and

Table 1 Comparison of clinical characteristics between PCDLBCL and SCDLBCL.

	PCDLBCL (n = 18)	SCDLBCL (n = 15)	*P*
Age at diagnosis, y, median (range)	75.5 (41–90)	73 (34–94)	.3354
Gender (%)			.1093
Male	7 (38.9)	10 (676.7)	
Female	11 (61.1)	5 (33.3)	
Anatomical skin location, n (%)			**.0040**
Leg	12 (66.7)	5 (33.3)	
Arm	2 (11.1)	1 (6.7)	
Head and neck	2 (11.1)		
Trunk	1 (5.6)		
More than 1 district	1 (5.6)	9 (60)	
Number of lesions, n (%)			.4814
Single	8 (44.4)	5 (33.3)	
Multiple	9 (50)	4 (26.7)	
Not available	1 (5.6)	6 (40)	
B symptoms, n (%)	1 (5.6)	6 (40)	**.0085**
First-line therapy, n (%)	Total N = 17	Total N = 13	.1148
Local: surgery and/or radiotherapy	3 (17.6)	0	
Systemic: R-CHOP or CHOP like	3 (17.6)	12 (92.3)	
Combined	11 (64.7)	1 (7.7)	
Response to first-line therapy, n (%)	Total N = 17	Total N = 12	**.0124**
CR	16 (94.1)	7 (58.3)	
PR	1 (5.9)	2 (16.7)	
No response/progression		3 (25)	
Cutaneous relapse, n (%)	6 (33.3)	7 (46.7)	.2462
Median time to relapse (range)	28.1 (7.6–73.2)	15.5 (1.9–51.9)	**.0216**

Abbreviations: PCDLBCL, primary cutaneous diffuse large B-cell lymphoma; SCDLBCL, secondary cutaneous diffuse large B-cell lymphoma; CR, complete response; PR, partial response.

differences in clinical presentation and in pathological and molecular features between PCDLBCL and SCDLCBL are reported in Tables 1 and S2.

3.2. Pathological features and immunohistochemical results

Overall, 36 skin biopsies and 9 extracutaneous biopsies were analyzed. Fig. 1 summarizes the pathological and immunophenotypical results observed in 18 PCDLBCL and 15 SCDLBCL cases. After pathologic review, the PCDLBCL cases were subclassified into PCDLBCL-LT (10 cases, 56%) and PCDLBCL-NOS (8 cases, 44%).

The application of the Hans' algorithm to PCLBCLs resulted in slightly more than half of the cases (10/18; 55.5%) classified as non-GC and the remaining as GC (8/18; 44.5%). Of these latter, 4 of 8 showed isolated expression of BCL6, and 4 of 8 expressed both BCL6 and CD10. PCDLBCL-LT as well as SCDLBCL cases mostly exhibited a non-GC phenotype (8/10, 80% and 10/15, 66.7%, respectively), whereas PCDLBCL-NOS mostly showed a GC phenotype (6/8, 75%; pV = 0.008). When BCL6, MUM1, and CD10 expression was considered separately, PCDLBCL and SCDLBCL cases showed a similar distribution of the positive cases. In PCDLBCL, BCL6 was expressed in 13 of 18 (72.2%), MUM1 in 12 of 18 (66.7%), and CD10 in 4 of 18 (22.2%) cases; similarly, in SCDLBCLs, BCL6 was expressed in 12 of 15 (80%), MUM1 in 10 of 15 (66.7%), and CD10 in 3 of 15 (20%) cases. In contrast, we found some trends in differences related to BCL2, MYC expression, and DE phenotype in PCDLBCL-NOS compared to both PCDLBCL-LT and SCDLBCL cases. In detail, BCL2 (which was not available in 1 case) was less expressed in PCDLBCL-NOS (4/8, 50%) than in PCDLBCL-LT cases (10/10, 100%) and in SCDLBCLs, independently from the COO (8/9, 88.8% of non-GC cases; 4/5, 80% of GC-cases; pV = 0.056). In addition, MYC (which was not available in 2 cases) was less expressed in PCDLBCL-NOS (2/7, 28.7%) than in PCDLBCL-LT (5/10, 50%) and in SCDLBCLs (2/5, 40% of GC cases and 5/9, 55.5% of non-GC cases; pV = 0.098). Finally, the DE phenotype was less frequently observed in PCDLBCL-NOS (1/7, 14.3%) than in PCDLBCL-LT (5/10, 50%), SCDLBCL-GC (2/5; 40%), and SCDLBCL-non-GC (4/9; 44.4%; pV = 0.097). High proliferation fraction (Ki-67 > 60%) was found in most DLBCL with cutaneous involvement, specifically with a median Ki-67 expression of 70% in PCDLBCL-NOS, 75% in SCDLBCL-non-GC, and 85% in PCDLBCL-LT and in SCDLBCL-GC. All data are reported in Table S2.

Comparison between PCDLBCL-NOS GC and the remaining PCDLBCL (ie, PCDLBCL-LT plus PCDLBCL-NOS non-GC) showed statistically significant differences in terms of median age at diagnosis (54.5 years versus 78 years, $P = .011$), presence of multiple skin lesions (0% versus 63%, $P = .007$), and rate of relapse (0% versus 55%, $P = .025$), thereby revealing a more indolent course.

3.3. COO classification by Lymph2Cx assay on NanoString platform

COO classification performed by NanoString technology failed in 5 cases and allowed classification of the remaining 28 lymphomas as ABC in 17 cases (8 PCDLBCL and 9 SCDLBCL), GC in 7 cases (5 PCDLBCL and 2 SCDLBCL), and unclassified in 4 cases (2 PCDLBCL and 2 SCDLBCL). Among PCDLBCL-LT cases, we did not find any GC, whereas the GC subtype was the most frequently observed (71.4%) among PCDLBCL-NOS cases.

COO classification by Lymph2Cx assay and Hans's algorithm was concordant in 18 of 28 evaluable cases (64.3%), of which 13 cases were classified as ABC/non-GC (5 PCDLBCL and 8 SCDLBCL) and 5 as GC/GC (4 PCDLBCL and 1 SCDLBCL). In contrast, discordancy was found in 4 cases classified as ABC by Lymph2cx assay and GC by Hans' algorithm, and in 2 cases classified as GC by Lymph2cx assay and non-GC according to Hans' algorithm. The 4 cases belonging to the unclassified group by Lymph2cx showed a non-GC phenotype by Hans' algorithm in 3 cases and a GC phenotype in the remaining one.

3.4. FISH analysis

FISH analysis was performed on 43 samples from 33 patients. Of these, 19 samples from 18 patients were PCDLBCL (in 1 case, we had the opportunity of analyzing both the primary lesion and its recurrence). The remaining 24 samples included 15 skin biopsies of patients with SCLBCL, as well as 9 biopsies of the primary extracutaneous sites that secondarily spread to the skin. Overall, 28 of the 43 samples (65.1%) belonging to 23 of the 33 patients (69.7%) harbored at least 1 gene rearrangement. Rearranged cases included 14 of 19 samples (66.6%) belonging to 13 of 18 patients (73.7%) with PCDLBCL and 15 of 24 samples (62.5%) belonging to 10 of 15 patients (66.6%) with SCLBCL (Table 2). All evaluable cases showed *IGH* rearrangement, whereas rearrangement of at least one of the *driver genes* (*BCL2*, *BCL6*, *MYC*) was detected only in 16 cases. In detail, *MYC* was the most frequently rearranged gene (13 samples from 11 patients, 5 with PCDLBCL and 6 with SCLBCL), followed by *BLC6* (8 samples from 7 patients, 4 with PCDLBCL and 3 with SCDLBCL). Finally, *BCL2* was rearranged in 5 samples from 3 patients, 1 with PCDLBCL and 2 with SCDLBCL. Intriguingly, in 8 samples from 7 patients (5 with PCDLBCL and 2 with SCDLBCL), FISH analyses documented isolated *IGH* rearrangements without identifying a putative partner of translocation. The comparison between the cytogenetic features of the first diagnostic biopsies and the recurrences in 10 patients revealed, in the patient with PCDLBCL and in 5 of 9 patients with SCDLBCL, a substantial overlap. Of the remaining 4 patients with SCDLBCL, 3 showed an additional gene rearrangement in the recurrences. In 1 case, the first biopsy showed the coexistence of *BCL6* and *IGH* rearrangements, whereas no gene rearrangements were found in the supposed recurrence, thus suggesting the possibility that the second lesion might be better interpreted as a new primary lesion than as a recurrence (Table 3). Co-existence of *BCL2*, *BCL6*, and/or *MYC* rearrangement in the same case was observed in 5 samples from 5 patients (2 with PCDLBCL and 3 with SCDLBCL). Interestingly, 3 of these cases were recurrences of previously diagnosed DLBCL that showed a lower number of rearranged genes at the first biopsy.

When PCDLBCL were subdivided into PCDLBCL-LT and PCDLBCL-NOS, 8 of 9 patients with PCDLBCL-LT had at least 1 gene rearrangement, whereas only 5 of 9 patients with PCLBCL-NOS were rearranged. Of these latter, 3 showed *MYC* gene rearrangements.

Finally, the majority of both primary (20/21) and secondary (14/15) DLBCL revealed polysomies in all the investigated regions.

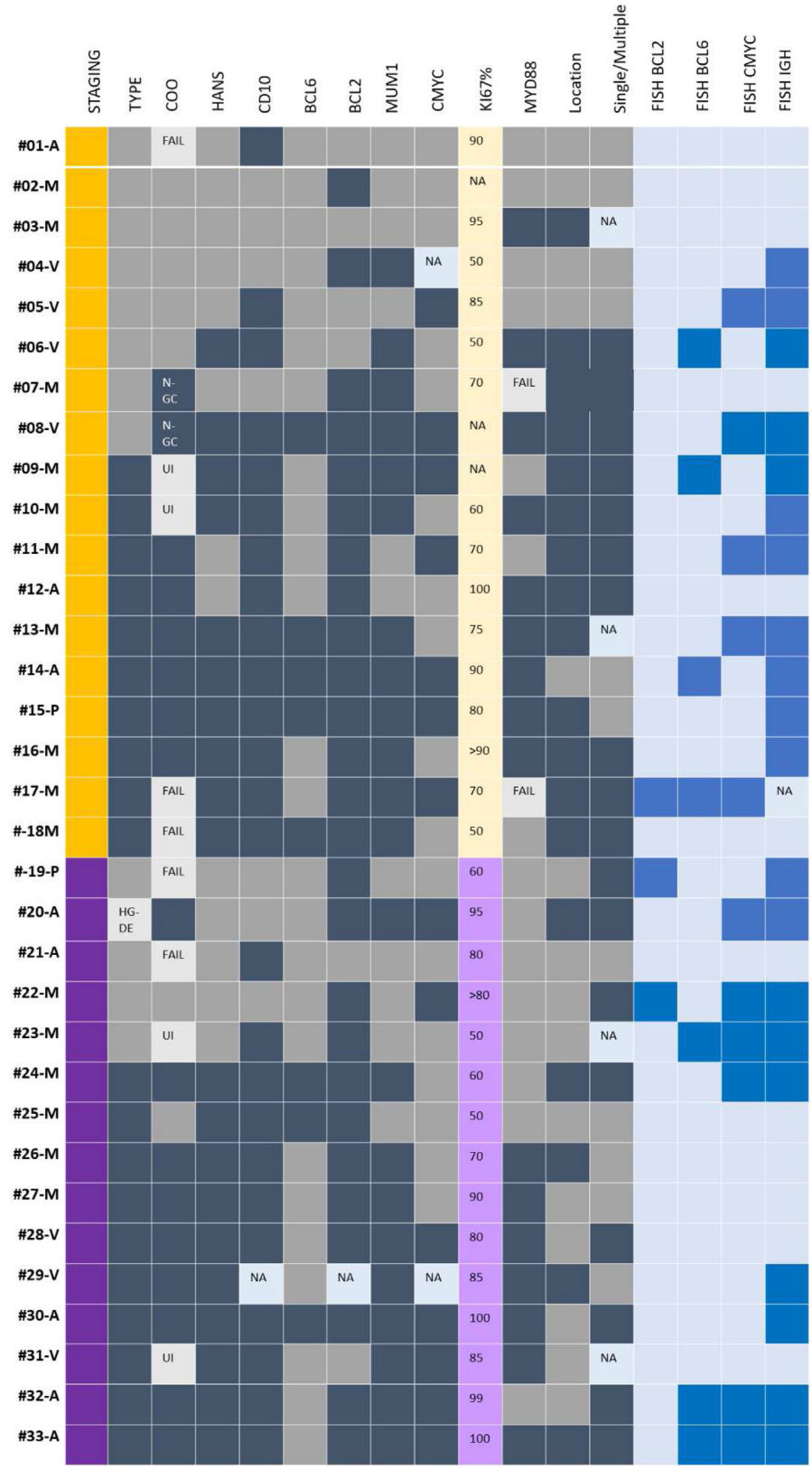
3.5. *MYD88* mutation analysis

Sequencing analysis showed an *MYD88* L265P mutation in 16 of 31 cases (51.6%), detected with a 6-90% VAF range; 15 cases resulted wild type (WT), and 2 cases failed. Among PCDLBCL cases, more than half of patients (9/16, 56.2%) harbored *MYD88* mutation, which was more frequently observed in PCDLBCL-LT (6/9, 66.7%) than in PCDLBCL-NOS (3/8, 37.5%). The median allelic burden of *MYD88* L265P was higher in PCDLBCL-LT (58.5%) than in PCDLBCL-NOS (28%) cases ($P = .0057$). Among SCDLBCL cases, *MYD88* mutation was found in 7 of 15 (46.7%), all belonging to the non-GC subtype.

Patients harboring *MYD88* mutations were older ($P = .0002$) and more frequently showed a non-GC phenotype according to Hans' ($P = .0023$), compared to patients with WT *MYD88*.

3.6. Survival analysis

PCDLBCL and SCDLBCL showed different survival outcomes, as expected (Fig. 2). The median OS was 99 months (95% confidence interval [CI] = 41-126) in PCDLBCL and 54 months (95% CI = 8-64) in SCDLBCL ($P = .0097$). PFS was also poorer in SCDLBCL compared to PCDLBCL (median PFS of 74 months and 15 months, respectively; $P = .0021$). Overall, survival analysis showed a significantly better outcome for *MYD88* WT CDLBCL


STAGING
TYPE
COO
HANS
CD10
BCL6
BCL2
MUM1
CMYC
KI67%
MYD88
Location
Single/Multiple
FISH BCL2
FISH BCL6
FISH CMYC
FISH IGH
#01-A
#02-M
#03-M
#04-V
#05-V
#06-V
#07-M
#08-V
#09-M
#10-M
#11-M
#12-A
#13-M
#14-A
#15-P
#16-M
#17-M
#-18M
#-19-P
#20-A
#21-A
#22-M
#23-M
#24-M
#25-M
#26-M
#27-M
#28-V
#29-V
#30-A
#31-V
#32-A
#33-A
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85
99
100

Table 2 FISH results of PCDLBCL and SCDLBCL positive for gene rearrangements.

Patient ID	Origin P/S	BCL6 % R-cell	MYC % R-cell	BCL2 % R-cell	IGH % R-cell
#09-M	P	41.3	N	N	18.5
#13-M	P	N	62.1	N	42.0
4#16-M	P	N	N	N	11.5
#16-M	P	N	N	N	20.2
#11-M	P	N	63.3	N	59.3
#10-M	P	N	N	N	11.5
#17-M	P	12.0	6.0	31.0	NA
#05-M	P	N	54.4	N	19.6
#08-V	P	N	71.1	N	80.4
#04-V	P	N	N	N	12.8
#06-V	P	6.7	N	N	9.1
#14-A	P	9.0	N	N	9.8
#15-P	P	N	N	N	14.0
#23-M	S	19.4	15.4	N	22.0
#24-M	S	N	70.2	N	66.7
#22-M	S	N	65.2	57.9	66.7
#29-V	S	N	N	N	31.9
#31-V	S	N	N	N	10.6
#32-A	S	84.0	13,3	N	83.0
#20-A	S	N	69.4	N	63.6
#33-A	S	11.4	85.3	N	81.0
#30-A	S	N	N	N	56.5
#19-P	S	N	N	19.1	43.4

Abbreviations: P, primary; S, secondary; R-cell, rearranged cells; N, negative for rearrangement; NA, not available; PCDLBCL, primary cutaneous diffuse large B-cell lymphoma; SCDLBCL, secondary cutaneous diffuse large B-cell lymphoma.

compared to the ones harboring the L265P mutation ($P = .0085$). Among PCDLBCL patients, the most relevant clinical prognostic factors in terms of both OS and PFS were age ($P = .0053$ and $P = .0049$, respectively) and *MYD88* mutation ($P = .0126$ and $P = .0028$, respectively). Among SCDLBCLs, only relapse and high Ki-67 proliferation index were associated with shorter OS ($P = .0060$ and $P = .0255$).

4. Discussion

This study aimed to compare the clinical, morphological, immunophenotypical, and molecular features of DLBCL with cutaneous involvement. We applied a comprehensive approach, including the evaluation of clinicopathological parameters, along with genetic and expression analyses, aiming to identify markers useful for risk stratification.

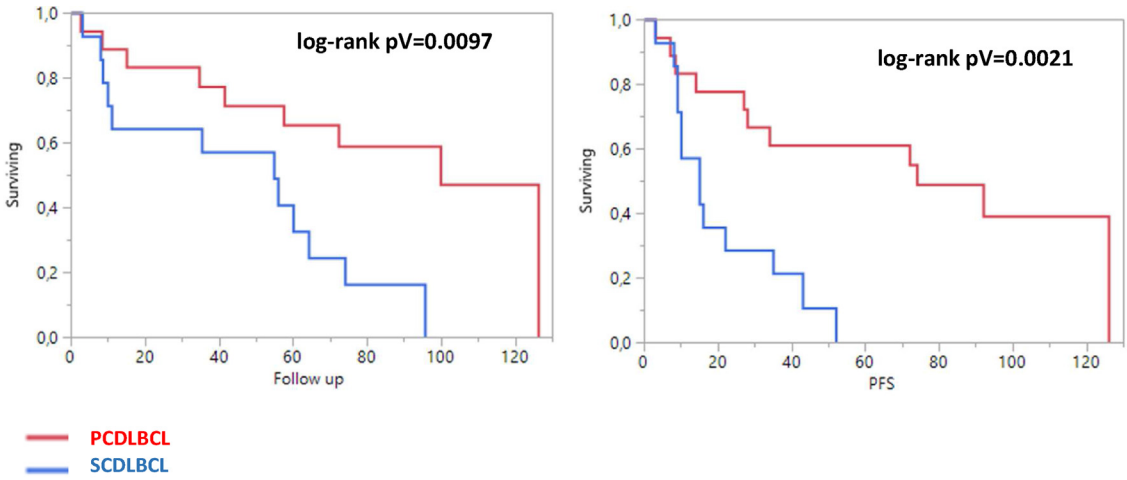
Our series included both primary cutaneous lymphomas and disseminated lymphomas also localized to the skin. This allowed us to compare the features of the 2 groups that, although exhibiting different outcomes, shared many biological similarities along with some important differences. Considering the COO, evaluated either with Hans's algorithm or with Lymph2cx assay, both PCDLBCL and SCDLBCL segregated with similar frequencies in the ABC/non-GC and the GC subtypes, with most of the cases belonging to the former. In our series, the ABC/non-GC cases frequently exhibited *MYD88* mutation, evoking the genetically defined MCD subtype of ABC DLBCL, which has a preferential extranodal tropism [7,8]. In contrast, GC cases rarely exhibited *MYD88* mutation. In turn, the frequency of gene rearrangements at FISH analysis was not related to the COO subtype, neither in PCDLBCL nor in SCDLBCL, but we found significantly higher numbers of gene rearrangements in the group of SCDLBCL, in which we found double-hit cases that were not present in the PCDLBCL group. This finding is not surprising, as the accumulation of gene rearrangement is not infrequently related to lymphoma progression and dissemination, thus to higher clinical stages and worse prognosis, as we described in extracutaneous sites [22–24]. In our study, the opportunity of analyzing samples from both initial lesions and recurrences of the same patients allowed us to confirm the paradigm of the genetic progression of high-grade B-cell lymphomas. Furthermore, we confirm the relevance of finding genetic markers of the disease in evaluating the differential diagnosis between the recurrences of a known lymphoma and the insurgence of a new one, ex novo. As a whole, and as expected, patients with SCDLBCL had a significantly shorter survival than those with PCDLBCL. In PCDLBCL patients, the most relevant clinical prognostic factors for OS and PFS were age and *MYD88* mutation, whereas in SCDLBCL, only relapse and high expression of Ki-67 were associated with a shorter OS. This observation confirms results already published by a Korean group on a series of 44 cutaneous DLBCLs (both primary and secondary). Even in that series, SCDLBCLs were characterized by a more advanced stage and a higher International Prognostic Index score than PCDLBC-LT. Interestingly, leg type demonstrated a better outcome than SCDLBCLs [25].

Fig. 1 Summary of clinicopathological, cytogenetic, and molecular features of the 18 patients with primary cutaneous diffuse large B-cell lymphomas (PCDLBCLs) and the 15 patients with secondary cutaneous diffuse large B-cell lymphomas (SCDLBCLs). **Staging:** yellow: PCDLBCL, purple: SCDLBCL**; Type:** grey: for PCDLBCL-NOS and SCDLBCL-GC; dark blue: for PCDLBCL-leg type (LT); for SCDLBCL-notGC; **COO:** grey: GC; dark blue: ABC; **HANS:** grey: GC; dark blue: not-GC; **CD10:** grey: positive; dark blue: negative; **BCL6**: grey positive; dark blue negative; **BCL2:** grey: negative; dark blue: positive; **MUM1:** grey: negative; dark blue: positive; **MYC:** grey: negative; dark blue: positive; **MYD88**: grey: not mutated; dark blue: L265P mutated; **Location:** grey: not leg; dark blue: leg; **Single/Multiple:**grey: single; dark blue: multiple; **FISH**: blue: rearranged; light blue: not rearranged. Abbreviations: NA, not available; U, unclassified; HG-DE, high-grade double expressor; N-GC, not germinal center.

Table 3 Comparative FISH results of different DLBCL in the same patients.

Patient ID	Sample	Site	Origin P/S	BCL6% R-cell	MYC % R-cell	BCL2% R-cell	IGH % R-cell
#16-M	Sample#1	Leg	P	N	N	N	N
	Sample#2	Leg	P	N	N	N	11.5
	Sample#3	Leg	P	N	N	N	20.2
#10-M	Sample#1	Leg	P	N	N	N	N
	Sample#2	Leg	P	N	N	N	11.5
#24-M	Sample#1	Nodal	SYS	N	67.0	N	72.3
	Sample#2	Leg	S	N	70.2	N	66.7
#25-M	Sample#1	Tonsil	SYS	48.0	N	N	51.2
	Sample#2	Chest	S	N	N	N	N
#22-M	Sample#1	Axillary nodes	SYS FL	N	N	57.6	59.7
	Sample#2	Lumbar	S	N	65.2	57.9	66.7
#26-M	Sample#1	Testicular	SYS	N	N	N	N
	Sample#2	Leg	S	N	N	N	N
#12-A	Sample#1	Nasopharyngeal	SYS	N	N	N	N
	Sample#2	Leg	P	N	N	N	N
#32-A	Sample#1	Iliopsoas muscle	SYS	77.3	N	N	74.8
	Sample#2	Shoulder	S	84.0	13.3	N	83.0
#20-A	Sample#1	Testicular	SYS	N	74.6	N	65.4
	Sample#2	Leg	S	N	69.4	N	63.6
#33-A	Sample#1	Nasopharyngeal	SYS	N	N	N	N
	Sample#2	Leg	S	11.4	85.3	N	81.0
#19-P	Sample#1	Mesenteric fat	SYS	N	N	51.1	71.6
	Sample#2	Abdomen	S	N	N	19.1	43.4

Abbreviations: P, primary; S, secondary; SYS, systemic; R-cell, rearranged cells; SYS FL, systemic follicular lymphoma; N, negative for rearrangement; DLBCL, diffuse large B-cell lymphoma.



	Median OS, months (95% C.I.)	Median PFS, months (95% C.I.)
PCDLBCL	99 (41-126)	74 (27-126)
SCDLBCL	54 (8-64)	15 (9-35)

Fig. 2 OS and PFS in PCDLBCL and SCDLBCL. Abbreviations: OS, overall survival; PFS, progression-free survival; PCDLBCL, primary cutaneous diffuse large B-cell lymphoma; SCDLBCL, secondary cutaneous diffuse large B-cell lymphoma; CI, confidence interval.

Focusing on PCDLBCL, the histopathological review, combined with the careful clinical study of each patient, was crucial for identifying the 2 separate entities of PCDLBCL-LT and DLBCL-NOS, which proved to have different clinicopathological and molecular profiles. In summary, PCDLBCL-LT, as a whole, confirmed to belong to the non-GC group defined by Hans [2] and, concordantly, to the ABC group identified by GEP. These lymphomas frequently showed BCL2 and MYC expression, having a so-called double expressor profile and frequently showed *MYD88* mutations. In contrast, such features were significantly less frequent in PCDLBCL-NOS, which, in turn, showed a peculiar morphology and immunophenotype, being composed predominantly of centroblasts intermingled with a variable reactive T lymphocytes and more frequently CD10-positive and BCL2-negative than PCDLBCL-LT. Survival analysis showed a significantly better patients' outcome in PCDLBCL-NOS than in PCDLBCL-LT in our series.

Overall, our data supported the existence of PCDLBCL-LT as an independent entity and suggested including the use of COO analysis to better classify the PCDLBCL-NOS cases, as recently highlighted [26] Our data are comparable with those from previous studies, including the NOS category more or less sharply separated from primary cutaneous follicle center with large cell predominance (PCFCL-LC) and from PCDLBCL-LT [19,20,25,27] and, particularly, with the study by Lucioni and colleagues [20] on a large Italian series of PCDLBCL with large-cell morphology, in which PCDLBCL-NOS were more frequent than PCDLBCL-LT (61% versus 39%). Our findings within a different Italian series are concordant in highlighting a relevant incidence of NOS cases by applying the same histopathological criteria (a diffuse proliferation of chiefly centroblastic cells intermingled with variable reactive CD3+ lymphocytes). Lucioni et al. [28] have also observed that cases within the NOS category may have an indolent clinical course and a better response to the radiotherapy, but can behave more aggressively when bearing a non-GC COO. In our study, we could not confirm these differences in survival or therapeutic response among NOS cases, nor differences regarding survival or incidence of MYC rearrangement between PCDLBCL-LT and PCDLBCL-NOS cases. A reasonable explanation might be the small size of the series. Instead, we found differences regarding the incidence of *MYD88* mutation, which resulted in a significant negative outcome predictor. Although several data, including our study, seem to sustain the existence of the NOS category, the debate is still ongoing. Leading researchers in the field of cutaneous lymphomas have not yet achieved a definitive consensus, including the authors of the updated WHO/EORTC classification who have deleted the NOS entity, previously called as *other* from the scheme [10,12]. According to them, only rare cases cannot be confidently classified among the PCFCL-LC or the PCDLBCL-LT, and the inclusion of cases to the NOS category might be subjective, potentially confusing, and detrimental in selecting the optimal treatment [16,29]. Conceivably, criteria used to identify such cases among lymphomas composed of large transformed cells are different among studies, considering either the morphologic appearance or phenotype, like negativity for BCL2 or positivity for CD10. Reproducibility of histologic criteria proposed by Lucioni [20] should be the object of future studies. From a technical point of view, our study confirms the imperfect correlation between COO determination by immunohistochemistry and GEP. Moreover, the COO classification by NanoString analysis seems to be essential to better classify PCDLBCL-NOS cases, bearing morphophenotypical atypical features. The data obtained with GEP performed with NanoString technology on PCDLBCL seems to be more similar to those reported by Cho and Hoefnagel [11,30], but significantly discordant with a more recent study by Schrader et al. [12] who have found a heterogeneous distribution of GC and ABC subtypes in PCDLBCL-LT and a homogeneous GC COO phenotype in the follicular lymphomas with large cells. From the clinical point of view, among PCDLBCL-LT cases, we did not find any GC case by COO; this might have some relevant therapeutic consequences, because to these cases we could add some Bruton Kinase Inhibitors, such as Ibrutinib [31], or lenalidomide [32], all effective in ABC DLBCL cases.

In conclusion, our study highlights clinicopathological, cytogenetic, and molecular similarities and differences between PCDLBCL and SCDLBCL, underlining the importance of staging at the time of diagnosis in cutaneous B-cell lymphoma. In the PCDLBC setting, we confirmed the independent clinicopathological entity of the leg-type category and discussed some open questions regarding the role of immunophenotype, COO, performed either by Hans's algorithm or by NanoString technology, genetic rearrangements of BCL6, BCL2, MYC, DH/TH status, and *MYD88* mutations, particularly in the NOS category. Further genetic and molecular researches will provide new ideas for exploring novel diagnostic, prognostic, and therapeutic targets in this field of pathology.

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R.P., S.G, and R.B. FISH analysis was performed by S.F. and M.G.T. Cytogenetic data interpretation was performed by M.G.T., F.M, S.F, and D.M. All authors edited and approved the final draft.

Data availability statement: The data supporting the findings of this study are available from the corresponding author (S.S.) upon request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2023.03.012.

References

[1] Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503−11. https://doi.org/10.1038/35000501.

[2] Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275−82. https://doi.org/10.1182/blood-2003-05-1545.

[3] Willemze R, Vergier B, Duncan LM. Primary cutaneous diffuse large B-cell lymphoma, leg type. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency Agency for Research on Cancer; 2017. p. 303−4.

[4] Yoon N, Ahn S, Yong Yoo H, Jin Kim S, Seog Kim W, Hyeh Ko Y. Cell-of-origin of diffuse large B-cell lymphomas determined by the Lymph2Cx assay: better prognostic indicator than Hans algorithm. Oncotarget 2017;8:22014−22. https://doi.org/10.18632/oncotarget.15782.

[5] Abdulla M, Hollander P, Pandzic T, et al. Cell-of-origin determined by both gene expression profiling and immunohistochemistry is the strongest predictor of survival in patients with diffuse large B-cell lymphoma. Am J Hematol 2020;95:57−67. https://doi.org/10.1002/ajh.25666.

[6] Bettelli S, Marcheselli R, Pozzi S, et al. Cell of origin (COO), BCL2/MYC status and IPI define a group of patients with Diffuse Large B-cell Lymphoma (DLBCL) with poor prognosis in a real-world clinical setting. Leuk Res 2021;104:106552. https://doi.org/10.1016/j.leukres.2021.106552.

[7] Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. N Engl J Med 2018;378:1396−407. https://doi.org/10.1056/NEJMoa1801445.

[8] Chen R, Zhou D, Wang L, Zhu L, Ye X. MYD88(L265P) and CD79B double mutations type (MCD type) of diffuse large B-cell lymphoma: mechanism, clinical characteristics, and targeted therapy. Ther Adv Hematol 2022;13:20406207211072840. https://doi.org/10.1177/20406207211072839.

[9] Kempf W, Zimmermann A-K, Mitteldorf C. Cutaneous lymphomas-An update 2019. Hematol Oncol 2019;37:43−7. https://doi.org/10.1002/hon.2584.

[10] Willemze R, Cerroni L, Kempf W, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. Blood 2019;133:1703−14. https://doi.org/10.1182/blood-2018-11-881268.

[11] Cho I, Yoon N, Hyeon J, et al. Comparison of the Lymph2Cx assay and Hans algorithm in determining the cell-of-origin of diffuse large B-cell lymphomas, not otherwise specified. Appl Immunohistochem Mol Morphol 2020;28:731−40. https://doi.org/10.1097/PAI.0000000000000843.

[12] Schrader AMR, de Groen RAL, Willemze R, et al. Cell-of-origin classification using the Hans and Lymph2Cx algorithms in primary cutaneous large B-cell lymphomas. Virchows Arch 2022;480:667−75. https://doi.org/10.1007/s00428-021-03265-5.

[13] Oschlies I, Wehkamp U. Cutaneous B cell lymphomas: standards in diagnostic and clinical work-up. Hints, pitfalls and recent advances. Histopathology 2022;80:184−95. https://doi.org/10.1111/his.14556.

[14] Sica A, Vitiello P, Caccavale S, et al. Primary cutaneous DLBCL non-GCB type: challenges of a rare case. Open Med (Warsaw, Poland) 2020;15:119−25. https://doi.org/10.1515/med-2020-0018.

[15] Pham-Ledard A, Beylot-Barry M, Barbe C, et al. High frequency and clinical prognostic value of MYD88 L265P mutation in primary cutaneous diffuse large B-cell lymphoma, leg-type. JAMA dermatology 2014;150:1173−9. https://doi.org/10.1001/jamadermatol.2014.821.

[16] Menguy S, Beylot-Barry M, Parrens M, et al. Primary cutaneous large B-cell lymphomas: relevance of the 2017 World Health Organization classification: clinicopathological and molecular analyses of 64 cases. Histopathology 2019;74:1067−80. https://doi.org/10.1111/his.13832.

[17] Menguy S, Gros A, Pham-Ledard A, et al. MYD88 somatic mutation is a diagnostic criterion in primary cutaneous large B-cell lymphoma. J Invest Dermatol 2016;136:1741−4. https://doi.org/10.1016/j.jid.2016.04.018.

[18] Lucioni M, Fraticelli S, Neri G, et al. Primary cutaneous B-cell lymphoma: an update on pathologic and molecular features. Hematology 2022;3:318−40. https://doi.org/10.3390/hemato3020023.

[19] Kodama K, Massone C, Chott A, Metze D, Kerl H, Cerroni L. Primary cutaneous large B-cell lymphomas: clinicopathologic features, classification, and prognostic factors in a large series of patients. Blood 2005;106:2491−7. https://doi.org/10.1182/blood-2005-03-1175.

[20] Lucioni M, Pescia C, Bonometti A, et al. Double expressor and double/triple hit status among primary cutaneous diffuse large B-cell lymphoma: a comparison between leg type and not otherwise specified subtypes. Hum Pathol 2021;111:1−9. https://doi.org/10.1016/j.humpath.2021.01.006.

[21] Tibiletti MG. Specificity of interphase fluorescence in situ hybridization for detection of chromosome aberrations in tumor pathology. Cancer Genet Cytogenet 2004;155:143−8. https://doi.org/10.1016/j.cancergencyto.2004.03.005.

[22] Bernasconi B, Uccella S, Martin V, et al. Gene translocations in testicular lymphomas. Leuk Lymphoma 2014;55:1410−2. https://doi.org/10.3109/10428194.2013.834055.

[23] Tibiletti MG, Martin V, Bernasconi B, et al. BCL2, BCL6, MYC, MALT 1, and BCL10 rearrangements in nodal diffuse large B-cell lymphomas: a multicenter evaluation of a new set of fluorescent in situ hybridization probes and correlation with clinical outcome. Hum Pathol 2009;40:645−52. https://doi.org/10.1016/j.humpath.2008.06.032.

[24] Magnoli F, Bernasconi B, Vivian L, et al. Primary extranodal diffuse large B-cell lymphomas: many sites, many entities? Clinicopathological, immunohistochemical and cytogenetic study of 106 cases. Cancer Genet 2018;228−229:28−40. https://doi.org/10.1016/j.cancergen.2018.08.001.

[25] Lee WJ, Won KH, Won CH, et al. Secondary cutaneous diffuse large B-cell lymphoma has a higher international prognostic index score and worse prognosis than diffuse large B-cell lymphoma, leg type. Acta Derm Venereol 2016;96:245−50. https://doi.org/10.2340/00015555-2139.

[26] Gros A, Menguy S, Bobée V, et al. Integrative diagnosis of primary cutaneous large B-cell lymphomas supports the relevance of cell of origin profiling. PLoS One 2022;17:e0266978. https://doi.org/10.1371/journal.pone.0266978.

[27] Felcht M, Klemke C-D, Nicolay JP, et al. Primary cutaneous diffuse large B-cell lymphoma, NOS and leg type: clinical, morphologic and

prognostic differences. J der Dtsch Dermatologischen Gesellschaft = J Ger Soc Dermatology JDDG. 2019;17:275−85. https://doi.org/10.1111/ddg.13773.

[28] Lucioni M, Berti E, Arcaini L, et al. Primary cutaneous B-cell lymphoma other than marginal zone: clinicopathologic analysis of 161 cases: comparison with current classification and definition of prognostic markers. Cancer Med 2016;5:2740−55. https://doi.org/10.1002/cam4.865.

[29] Cerroni L. Cutaneous diffuse large B cell lymphoma, leg-type. In: Skin lymphoma: the illustrated guide. John Wiley & Sons; 2020. p. 299−314.

[30] Hoefnagel JJ, Vermeer MH, Jansen PM, et al. Primary cutaneous marginal zone B-cell lymphoma: clinical and therapeutic features in 50 cases. Arch Dermatol 2005;141:1139−45. https://doi.org/10.1001/archderm.141.9.1139.

[31] Wilson WH, Wright GW, Huang DW, et al. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. Cancer Cell 2021 13;39:1643−1653.e3. https://doi.org/10.1016/j.ccell.2021.10.006.

[32] Goldfinger M, Cooper DL. Lenalidomide in DLBCL: are we past the cell of origin? Clin Adv Hematol Oncol 2021;19:320−5. PMID: 33989279.