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High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements: Biopsy analysis of 70 cases from the Slovak Lymphoma Registry

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We performed a twelve-year retrospective analysis of diffuse large B-cell lymphoma (DLBCL) patients' biopsies with rearrangements of genes MYC, BCL2, and/or BCL6, commonly referred to as double-hit and triple-hit high-grade B-cell lymphomas (DH/TH HGBL). Our aim was to present complex characteristics of the DH/TH HGBL group of patients diagnosed in the Slovak National Lymphoma Register together with the evaluation of the relationship between immunohistochemical (IHC) protein expressions of c-myc, bcl2, bcl6, and cyclin D1 in tissue specimens and the presence of rearrangements of their protein-coding genes by FISH analysis in order to find a clinically relevant diagnostic algorithm that would be the most time- and cost-efficient. For this study, a standard panel of histomorphological, IHC, and FISH methods was used to analyze the characteristics of 70 DH/TH HGBL patients' biopsies. Our study showed a predominance of the immunohistochemical GCB subtype over the non-GCB subtype (59:10 cases) in DH/TH lymphomas. The centroblastic morphology was the most commonly observed (30/70 cases; 43%). Furthermore, our study showed a high predominance of DH lymphoma cases with simultaneous MYC and BCL2 genes rearrangements (40/70; 57%), followed by an almost equal incidence of DH lymphomas with rearrangements of MYC and BCL6 genes (16/70; 23%) and of TH lymphomas (14/70; 20%). 15 of 16 FISH-examined DLBCL cases were negative for CCND1 rearrangement. A great majority of DH/TH cases showed also immunohistochemical overexpression of corresponding proteins (62/70; 89%), mostly in a form of triple expressor of c-myc/bcl2/bcl6 proteins (36/70; 51%), followed by c-myc/bcl2 and c-myc/bcl6 double expressor proteins positivity (20/70 and 6/70, respectively). Comparing preferential FISH testing of DE/TE and GCB DLBCL cases for genetic rearrangements we would be able to detect 89% and 84% of our HGBL-DH, TH group of patients, respectively. None of the examined methods for economically rational FISH testing showed enough concordance with IHC analysis results. We might, therefore, advocate the complex testing of all DLBCL patients' biopsies including FISH analyses.

Key words: double-hit/triple-hit, DLBCL, high grade B-cell lymphoma, double expressor/triple expressor, GCB/non-GCB

Diffuse large B-cell lymphomas, not otherwise specified (further referred to as DLBCL) are the most common malignant lymphomas in the Caucasian population, comprising approximately 35% of all non-Hodgkin lymphomas [1]. Biologically they represent a non-homogenous group of tumors with distinctive morphology, immunological and genetic profile, clinical presentation, and survival. Therefore, with the utilization of an increasing amount of information about their cancerogenesis, new attempts for clinically significant stratification continually emerge. For analysis of biopsy specimens of patients with DLBCL, histological and immunohistochemical (IHC) analyses gradually started to be used followed later on by DNA analyses using the fluorescence *in situ* hybridization (FISH) method. Other methods such as analyses of DNA and RNA isolated from the tumor sample and/or blood plasma of the patient were added recently.

With the development of new treatment modalities, the original histopathological identification of DLBCL morphological variants lost its prognostic significance. On the contrary, an identification of the histogenetic origin of the tumor cells using gene expression profiling, so called COO (cell-of-origin) concept became clinically significant. According to the concept, it is relevant to distinguish GCB (Germinal Centre B-Cell) subtype from ABC (Activated B-Cell) subtype, the latter being associated with a worse

prognosis of the patients [2, 3]. However, in real practice, this procedure is too demanding and is often replaced by IHC analysis of different proteins expression in tumor cells summarized into various binary algorithms. The most popular and commonly used is the Hans algorithm allowing to discern GCB versus non-GCB DLBCL subtype [4].

Other possibilities of relevant DLBCL prognostic stratification came with the implementation of molecular-cytogenetic fluorescence *in situ* hybridization analyses of genetic alterations that play a role in the pathogenesis of this lymphoma. For instance, *MYC* gene rearrangement in DLBCL represents a negative prognostic parameter [5]. The presence of concurrent rearrangements of the *MYC* gene and at least one of two other genes *BCL2* and/or *BCL6* enables to identify a rare subset of DLBCL cases traditionally called double-hit and triple-hit lymphomas (further referred to as DH and TH). DH lymphomas are defined by the concurrent translocations either in genes *MYC* and *BCL2* or *MYC* and *BCL6*, TH lymphomas have translocated all three genes *MYC*, *BCL2,* and *BCL6*. DH and TH are characterized by a more aggressive clinical course, poor treatment response, often extranodal localization of the tumor, and short median overall survival of 5–18 months [6–9]. Therefore, nowadays they are classified as a separate group named high-grade B-cell lymphomas (HGBL) with given gene rearrangements [1, 10]. Taking into account the incidence of DLBCL, the high economic and time burden of molecular-cytogenetic examinations again led to an attempt to simplify testing using IHC analyses. Since the majority of DH/TH lymphomas are at the same time double expressors or triple expressors of corresponding proteins (DE, TE) by phenotype [11], assessing for c-myc, bcl2, and/or bcl6 protein expression could accelerate and simplify also patients' stratification into the biologically distinctive subgroups.

Another potential IHC prognostic marker of DLBCL is an aberrant CD5 positivity, rarely accompanied by the co-expression of cyclin D1 and/or SOX11 proteins [12], in which case there is a need for FISH analysis of *CCND1* translocation to distinguish blastoid variant of mantle cell lymphoma from DLBCL [13].

In our retrospective analysis, we have aimed to find the relation between economically more convenient IHC procedures that would help us with the identification of more aggressive types of DLBCL lymphomas compared to traditional FISH analyses. Therefore, we have analyzed the relationship of the protein expressions (cyclin D1, c-myc, bcl2, and bcl6) in tissues and the presence of rearrangements of their protein-coding genes diagnosed in a large number of DLBCL bioptic specimens. All that might allow to verify the effectiveness of the implementation of molecular cytogenetic tests into the daily clinical practice. Identification of HGBL with double and/or triple rearrangements is currently important not just for their dismal outcome in comparison with DLBCL patients without these multiple rearrangements, but mostly due to the actually discussed necessity of using different therapeutic modalities in the DH/TH group of patients [14].

Patients and methods

Patient characteristics and methodology of the study. Biopsy specimens of patients with DLBCL from various hospitals in the Slovak Republic were collected over the period of 12 years in the Slovak National Lymphoma Register. For this study, we reanalyzed all the cases, in which the biopsy diagnostic analysis included FISH testing for rearrangements in *MYC, BCL2, BCL6,* and/or *CCND1* genes. The indication criteria for FISH analysis were as follows: a) the tumor showed atypical blastoid or Burkitt-like morphology and/or atypical phenotype including CD5 positivity, and b) the testing was required by the clinical oncologist to determine the DH/TH genotype. Analyzed clinicopathological parameters of patients included medical history to distinguish primary versus secondary DLBCL, localization of the lymphoma to distinguish nodal versus extranodal localization of the lymphoma, and the patient's age and gender.

The histological and IHC microscopical slides were retrospectively evaluated by two reviewers (KL and LP). First, we analyzed the relationship between tumor morphological type and the gene rearrangement patterns. Following the recent WHO classification [1], the cases were categorized into histomorphological groups with centroblastic, immunoblastic, anaplastic, blastoid, or Burkitt-like morphology. Second, we evaluated the relationship between the results of FISH analyses and the results of IHC analyses in the categories of $CD5^+$, GCB, and/or non-GCB DLBCL subtypes. Third, the analysis included also the data on the age and gender of the patients as well as data on nodal versus extranodal manifestation of the disease. Lastly, the lymphoma biopsy register was reviewed for staging bone marrow (BM) biopsies of the patients.

Histological and IHC methods. Formalin-fixed, paraffin-embedded tissues were analyzed with a standard lymphoma panel of histological staining (H&E and Giemsa staining, PAS reaction, and silver Gomori impregnation). All of the cases were examined immunohistochemically (either at the time of diagnosis or retrospectively for this study) with the usage of a panel of the antibodies for: a) confirmation of the DLBCL diagnosis (antibodies to CD20, PAX5, CD3, CD5; in case of CD5 positivity also cyclin D1), b) verification of GCB versus non-GCB phenotype using the Hans algorithm (antibodies to CD10, bcl6, MUM1) [4], c) identification of bcl2 [15] and c-myc protein expression. The list of the immunostains used together with their detection methods is summarized in Table 1.

Examination of c-myc expression was performed manually by standard deparaffinization of the tissue, antigen retrieval using EDTA reagent in a water bath set to 97 °C for 45 min, then by the staining with the use of monoclonal antibody c-myc/EP121 (Bio SB, 1:50) for 45 min and finally

Table 1. Antibodies used for IHC analysis of DH and TH lymphomas with their detection methods.

	Name of the antibody/clone	Catalog number	Producer	Dilution	Staining	Detection system
a)	CD 20/L26	IR 604	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
	CD 3	IR 503	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
	CD 5/4C7	IR 082	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
	PAX5/DAK-Pax5	IR 650	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
	cyclin D1/EP12	IR 083	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
	CD10/56C6	IR 648	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
b)	Bcl2/124	IR 614	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
	Bcl6/PG-B6p	IR 625	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
	MUM1/MUM1p	IR 644	Dako	RTU	Autostainer Link 48	EnVision FLEX/HRP
c)	c-myc/EP121	BSB 6581	Bio SB	1:50	Manually	EnVision FLEX/HRP

Abbreviations: RTU: ready-to-use, HRP: horseradish peroxidase

applied detection system EnVision FLEX/HRP, DAB and contrast hematoxylin staining. After deparaffinization, all of the other antibody clones (FLEX, Dako) were processed using the automated immunohistochemistry platform PTLink (Dako, Denamrk A/S, Glostrup, Denmark) and revitalized in high pH solution (pH 9) or low pH solution (pH 6.1), the latter being applied to PAX5 antibody, during 20 min at the temperature of 97 °C. The subsequent immunohistochemical reaction took place using Autostainer Link 48 (Dako, Denmark). Visualization was performed using EnVision FLEX/HRP (Dako), DAB (EnVision FLEX, Dako), and contrast hematoxylin staining (EnVision FLEX, Dako). A cut-off of ≤40% positive tumor cells has been suggested for the standardized evaluation of the specimens with an intranuclear protein expression following the criteria given by de Jong et al. [1, 16].

Fluorescence *in situ* hybridization. After the standard deparaffinization of biopsy sections, denaturation of the probes (ZytoLight SPEC MYC/BCL2/BCL6/CCND1 Dual Color Break Apart Probe, Zytovision) was performed in a hybridizer (DAKO Denmark) at the temperature of 73 °C for 5 min, followed by the overnight hybridization during 16 h at 37 °C and subsequently the specimens were examined under the fluorescence microscope. The presence of translocations involving *MYC* (8q24), *BCL2* (18q21), *BCL6* (3q27), and *CCND1* (11q13) genes was evaluated, each of them in 100 interphase nuclei. Using the break-apart probe the tumor cells were considered positive for gene rearrangement when a pair of green and red signal was separated by a distance greater than the doubled radius of a larger signal. At least 5% of examined cells needed to express such chromosomal changes in order to consider the case to be positive for given gene translocation [17].

The algorithm for DH/TH FISH analysis has changed over the monitored time period. In the first 10 years of the period, the FISH analyses of *MYC, BCL2,* and *BCL6* genes were performed simultaneously. In 2017, we started to examine at first *MYC* gene rearrangement and for cases without *MYC* translocation, the FISH analysis for *BCL2* and *BCL6* rearrangements were not realized. In addition, each CD5 and/or cyclin D1 positive case by IHC was examined for the *CCND1* gene rearrangement.

Results

Overview of the examination results. In the analyzed twelve-year period, the diagnosis of DLBCL was verified in biopsy specimens of 2,463 patients, and in 593 of them (24.1% of all) the diagnostic procedure included also various FISH analyses (Figure 1).

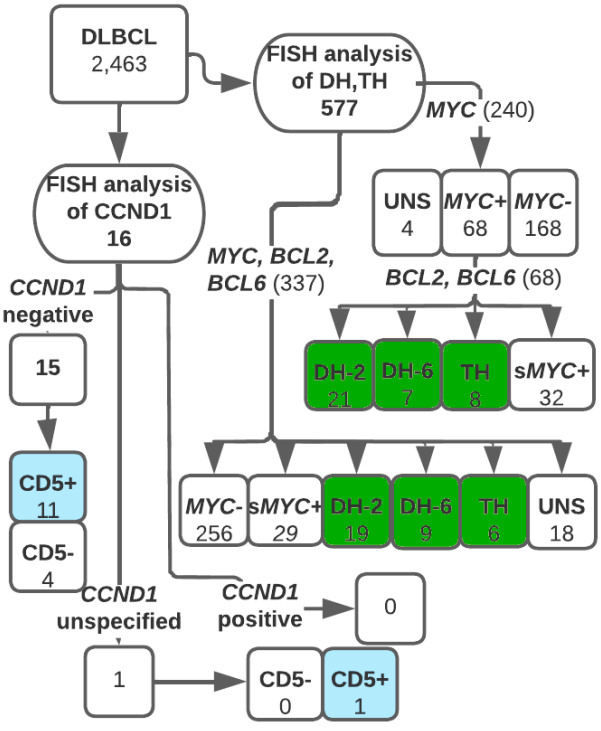

Figure 1. Diagram showing results of FISH analyses of DLBCL cases. Legend: blue boxes – CD5 positive DLBCLs which were analyzed for *CCND1* rearrangement; green boxes – double-hit and triple-hit lymphomas identified by FISH; Abbreviations: UNS – *MYC* unspecified; s*MYC*- single *MYC* rearrangement

FISH examination to detect DH/TH genotype was included in the biopsy evaluation of 577 cases (23.5% of all DLBCL examined by the FISH method). The procedure for a simultaneous examination of *MYC*, *BCL2*, and *BCL6* gene alterations was applied in 337 of them. In another 240 cases, the *BCL2* and *BCL6* genes were examined only after the confirmation of the *MYC* gene rearrangement. In addition, in 16 cases (0.6% of all DLBCL) the diagnostic algorithm included exclusively FISH analysis for the *CCND1* gene status, either because of immunohistochemical CD5 expression (12 cases) and/or partial cyclin D1 expression (4 cases). This FISH examination did not prove the *CCND1* gene rearrangement in 15 cases, in one case the examination could not be evaluated due to artifacts preventing correct signal visualization in lymphoma cells.

Clinicopathological characteristics of DH/TH lymphomas. Altogether, the concurrent rearrangements of *MYC*, *BCL2*, and/or *BCL6* genes by FISH was identified in biopsies of 70 patients (48 females and 22 males). Median age of patients was 66 years (mean age 63.6 years old), ranging from 12 to 87 years. At the time of diagnosis, 69% of them were at least 60 years old and just 2 patients were <30 (12 and 16) years old.

46% of DH/TH lymphomas (32 cases) were found in nodal biopsies and 57% (40 cases) in the extranodal manifestations (incl. simultaneous biopsies of nodal and extranodal DLBCL manifestation of two patients). According to patients' medical history, 11 of 70 DH/TH lymphomas represented secondary tumors, including 7 patients showing the transformation from primary follicular lymphoma. The others were diagnosed in patients with other malignant lymphoma types: in 2 patients with primary marginal zone B-cell lymphoma, 1 with chronic lymphocytic lymphoma, and in one with primary classical Hodgkin lymphoma. By review of our register, we identified BM staging biopsies of 51 patients from the 70 DH/TH cohort. These BM biopsies showed lymphoma involvement in 16 cases: 10 patients showed identical DLBCL infiltration as in the primary biopsy, while the other 6 patients presented discordant lymphoid infiltrates consisting of small B-cell lymphoma morphology exclusively. The BM staging biopsies of 35 patients were without any lymphomatous involvement.

In regard to the histomorphological subtypization of analyzed DH/TH cases, the centroblastic morphology was observed in 30 cases (43%), and other cases showed immunoblastic, anaplastic, blastoid, and Burkitt-like morphology in 4, 2, 23, and 6 cases, respectively (Figures 2A–2F). Due to quantitative and technical limits of the tissue specimens, 5 cases were considered to be morphologically unclassifiable.

The overview of all FISH verified 70 DH/TH lymphomas and their relation to IHC verified DE/TE phenotype is summarized in Table 2 and Figure 3. The double-hit status was identified in 56 of 70 cases (80%), it was represented by concurrent rearrangement of *MYC* and *BCL2* genes in 40 cases (57%), and by double rearrangement of *MYC* and *BCL6* genes in 16 cases (23%). The triple-hit status consisting of simultaneous rearrangement of all three *MYC*, *BCL2*, and *BCL6* genes was detected in 14 cases (20%).

Immunohistochemically, 62 of 70 (89%) DH/TH cases showed positivity of c-myc, bcl2, and/or bcl6 proteins allowing to classify them as double-/triple-expressors as follows: TE phenotype was identified in 36 patients (51%) and DE in 26 cases; 20 (29%) with simultaneous c-myc plus bcl2 positivity versus 6 (9%) with c-myc plus bcl6 proteins positivity (Figure 4). Four cases showed the phenotype neither of double nor triple expressor. In 4 cases it was not possible to trace back and investigate the IHC expression of the c-myc protein, thus they were not assigned to any of the DE/TE categories.

Using the Hans algorithm, the predominance of the GCB subtype (59 cases) over the non-GCB subtype (10 cases) at a ratio of 6:1 was identified in the analyzed cohort of DH/TH

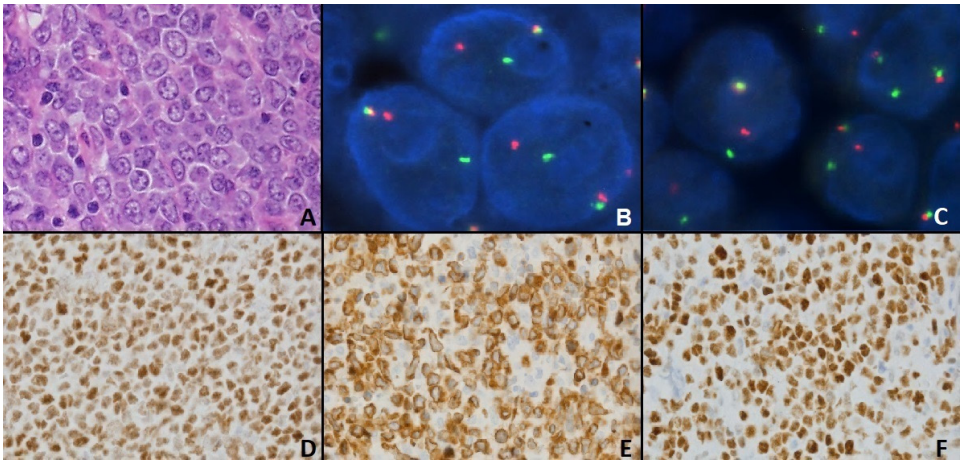

Figure 2. HGBL of DH and TE type. HGBL of DH and TE type with A) centroblastic morphology, H&E stain, 40× magnification; B) *MYC* gene rearrangement; and C) *BCL6* gene rearrangement (both by FISH analysis, 100× magnification with immersion oil); D) c-myc protein overexpression' E) bcl2 protein overexpression' and F) bcl6 protein overexpression (all demonstrated by IHC at 20× magnification).

Table 2. Summary of DH, TH lymphoma characteristics, including DE, TE status, GCB vs. non-GCB subtype and morphological variants.

	DH-2 (*MYC* and *BCL2*) 40 cases (57%)	DH-6 (*MYC* and *BCL6*) 16 cases (23%)	TH (*MYC*, *BCL2*, and *BCL6*) 14 cases (20%)	All DH, TH 70 cases (100%)
DE-2 (c-myc and bcl2)	11 (16%)	5 (7%)	4 (6%)	20 (29%)
DE-6 (c-myc and bcl6)	2 (3%)	4 (6%)	0 (0%)	6 (9%)
TE (c-myc, bcl2, and bcl6)	22 (31%)	4 (6%)	10 (14%)	36 (51%)
non-DE, TE	2 (3%)	2 (3%)	0 (0%)	4 (6%)
unclassified	3 (4%)	1 (1%)	0 (0%)	4 (6%)
GCB	39 (56%)	9 (13%)	11 (16%)	59 (84%)
non-GCB	1 (1%)	6 (9%)	3 (4%)	10 (14%)
unclassified	0 (0%)	1 (1%)	0 (0%)	1 (1%)
centroblastic	16 (23%)	6 (9%)	8 (11%)	30 (43%)
immunoblastic	3 (4%)	1 (1%)	0 (0%)	4 (6%)
anaplastic	2 (3%)	0 (0%)	0 (0%)	2 (3%)
blastoid	14 (20%)	4 (6%)	5 (7%)	23 (33%)
Burkitt-like	4 (6%)	2 (3%)	0 (0%)	6 (9%)
unclassified	1 (1%)	3 (4%)	1 (1%)	5 (7%)

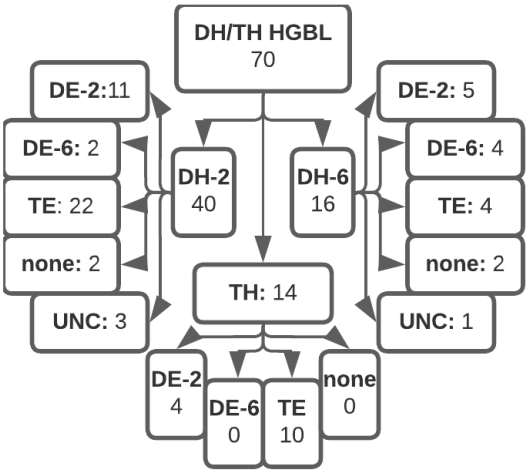

Figure 3. Distribution of double/triple protein expression positivity within DH/TH lymphomas. Abbreviations: UNC – unclassified; none – without double-/triple-expressor phenotype

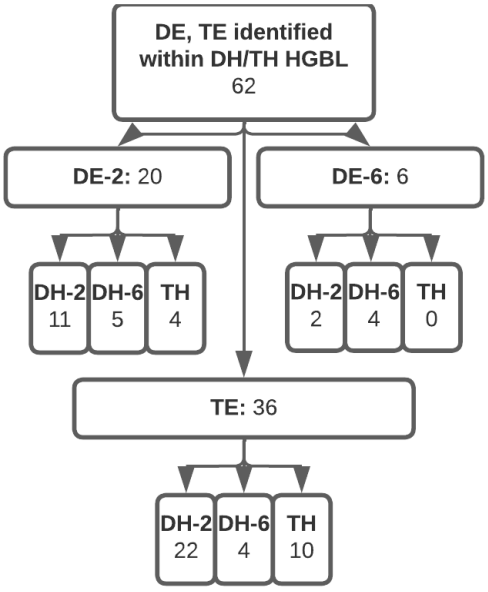

Figure 4. Distribution of double/triple-hits within DH/TH HGBL with concurrent double/triple expression of c-myc, bcl2, and/or bcl6 proteins.

lymphomas, while in one case the phenotypic stratification was not possible due to the technical reasons. The DH/TH lymphomas which were at the same time classified as DE or TE tumors belonged predominantly to GCB (52 cases) and less often to the non-GCB subtype (9 cases) at a ratio of 6:1.

Discussion

Despite adding rituximab to the CHOP regimen at the beginning of the millennium leading to significant improvement in DLBCL treatment outcomes, approximately 10–15% of DLBCL patients have primary refractory disease and an additional 20–25% develop relapse after an initial response, typically within first 2 years [18]. In addition, up to 1/3 of patients will have the primary refractory disease even with intensified chemoimmunotherapy regimens such as DA-EPOCH-R, R-hyper-CVAD/MA, or R-CODOX-M/R-IVAC [9]. Considering the high numbers of newly diagnosed DLBCL cases per year, this group of patients with critically low overall survival and inadequate response to treatment, nowadays known as HGBL, NOS, and DH/TH HGBL, represent an unmet need for understanding a specific background of the biological aggressivity of the disease and a proper both diagnostic and therapeutical management of such patients.

That was for us the reason to investigate a series of double-hit and triple-hit diffuse large B-cell lymphoma patients' biopsies diagnosed in the Slovak National Lymphoma

Register within a 12-year period. For the study, a standard panel of histomorphological, IHC, and FISH methods was used in regard to analyze the characteristics of the patients and to find the time- and cost-effective, and clinically relevant diagnostic algorithm.

Over this period, the FISH analyses were used under predefined restricted selection criteria, reflecting histomorphology of the tumor and/or clinical requirements, which might have impacted some obtained parameters. Our series showed two times more DH/TH HGBL lymphomas in female than in male patients (a ratio of 2.2:1), in contrast to literary known almost equal affection of both genders, or sometimes with a slight DH/TH HGBL predominance in males [1]. On the contrary, the age distribution of DH/TH HGBL patients in our study does not considerably differ from the usual age distribution of the DH/TH HGBL group of patients, as the reported median age of these patients at the time of diagnosis is in the 6th to 7th decade [1]. With regard to clinicopathological characteristics typically displayed by more aggressive non-Hodgkin lymphomas, we have also observed more often extranodal than nodal localization of the disease. Evaluation of available staging trephine biopsies showed a relatively high tendency of the disease to disseminate into the bone marrow. The positive staging bone marrow biopsies showed either concordant blastic or discordant small B-cell infiltration; the second was true for cases with verified high-grade secondary transformation from antecedent indolent lymphoma, typically from previously diagnosed follicular lymphoma.

In concordance with previous studies, our series showed a high predominance of DH lymphoma cases with simultaneous *MYC* and *BCL2* genes rearrangements [19, 20], followed by the almost equal incidence of DH lymphomas with rearrangements of *MYC* and *BCL6* genes and of TH lymphomas. Similarly, to the published results [11, 21], a great majority of DH/TH cases showed also immunohistochemically the overexpression of corresponding proteins, mostly in a form of a triple expressor of c-myc, bcl2, and bcl6 protein positivity. This double or triple expression is considered to have a negative prognostic impact on the overall survival of DLBCL patients, although the outcome is still better than in patients with identified the DH/TH rearrangements by FISH analysis, indicating that the higher aggressivity of DH/TH lymphomas may also be caused due to other potential reasons than gene rearrangements [22, 23].

All of this information suggests that the rationale for economical DLBCL FISH testing could start with a preferential examination of DE/TE DLBCL lymphomas for possible gene rearrangements. However, as stated by Friedberg (2017), up to one-third of DLBCL are double expressors by immunophenotype, but just approximately 10% of DLBCL are double-hits by genetic FISH analysis. Results of our study show that by preferential testing of DE/TE cases for gene rearrangements we would be able to detect 89% of our DH/TH HGBL cases and would miss the cases without expression of double/triple immunophenotype by IHC.

Furthermore, we have verified that the GCB phenotype proved by the Hans IHC algorithm [4] in more than 80% of the cases represents the most common subtype found in DH/TH HGBL. On the contrary, DE/TE which do not show DH/TH status were almost evenly distributed between both GCB and non-GCB subtypes, the latter being slightly predominant. This may imply a question of whether preferential FISH testing of GCB DLBCL lymphomas would be more economically beneficial in order to verify double/triple rearrangements than genetic testing of all DLBCLs. However, here again the obtained data of our study allow concluding that by preferential testing of DLBCL with the GCB phenotype for gene rearrangements we would be able to detect just 84% of our DH/TH HGBL cases and would miss the detection of DH/TH profile in biopsies being classified as non-GCB subtypes by IHC.

In regard to the histomorphological subtypization of analyzed DH/TH cases, as expected, the centroblastic morphology was the most common followed by two variants with atypical morphology (blastoid and Burkitt-like), while immunoblastic and anaplastic morphologies were observed quite sporadically. According to recent studies [24], the two atypical morphological variants are suggestive of more aggressive biological behavior. These data were not yet known during our study, however recently became to be a subject of change for a redefinition of our criteria indicative for the FISH examinations.

In addition, the co-testing of alterations of the CCND1 gene by FISH seems to be justified not only in CD5 and/or cyclin D1 immunohistochemically positive cases to exclude a diagnosis of blastoid MCL, but also in relation to very recent data on the possible identification of simultaneous rearrangement of the CCND1 and MYC genes leading to the problems of the classification and assessment of the biological behavior of such case [25].

We may summarize, that although NGS analyses of tumor DNA and RNA obtained from the tissue and or liquid biopsies are on the horizon, at the moment they seem to be used mostly in clinical studies. In contrast, the results of the FISH analyses are to be utilized in the real clinico-oncological practice. In optimal conditions and in agreement with the principles of personalized and precise medicine we might advocate the complex testing of all DLBCL patients' biopsies including FISH analyses. Such an approach allows the responsible hematooncologists to select the most proper treatment for every individual patient with DH/TH HGBL.

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