Clinicopathological correlations of the immunoprofile in diffuse large B-cell lymphoma NOS - a single institution's experience

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SUMMARY

The experimental platform in hematooncology is still searching for more valid prognostic and predictive factors on clinical, morphological and molecular levels. The bridge closer to daily practice is so-called translation medicine and from this point of view we have tried to sort diffuse large B-cell lymphoma not otherwise specified. The applied methodological approaches are morphology, indirect immunohistochemistry on formaline-fixed, parrafin-embeded tissue, Hans classifier sorting, expression of Bcl-2, CD5, CD20, CD30 and NfκB proteins in comparison with the clinical (Ann Arbor stage, IPI, aa-IPI, PFS, OS), laboratory and cytogenetic results (complex and simplex karyotypes). Statistical analysis included Cox regressive analysis, Mann-Whitney and Kruska-Wallis test. The interval of PFS and OS has been assessed according to Kaplan-Meier analysis. According to Hans classifier 11 cases (18.7 %) could not be sorted exactly into GCB/nonGCB-like subgroups. All relapsing cases bear negative expression of CD10 and 28 cases of non-relapsing cases showed positive expression of CD10. The "third" - GCB-like/nonGCB-like unsortable subgroups shared a very similar course of PFS with the nonGCB-like subgroup and a worse clinical course of OS. Statistically nonsignificantly better response to chemotherapy was shown by cases with positive Bcl-2 expression of more than 30 %. Statistically nonsignificantly better OS and PFS was shown by cases with a proliferation index Ki67 more than 70 %. The study detected 17 cases (28.8 %) with a nuclear expression of p50 and one case with nuclear expression of p65 (1.7 %) which may imply the possibility of NfκB signaling pathway activation. A statistically nonsignificant realationship of p50 expression and OS/PFS was indicated.

Keywords: diffuse large B-cell lymphoma – classification – prognosis – immunohistochemistry – NfκB signaling pathway – FFPE tissue.

Klinicko-patologická korelace imunoprofilu u difúzního velkobuněčného lymfomu, NOS - zkušenost z jednoho pracoviště

SOUHRN

Experimentální hematoonkologie zrcadlí potřebu hledání a identifikace nových validních prognostických a prediktivních faktorů na úrovni klinické, morfologické a molekulárně biologické. Translační medicína představuje tu část experimentální lékařské vědy, která je nejblíže běžné rutinní praxi hematoonkologie, a v její vizi jsme se pokusili odlišit a třídit difuzní velkobuněčný B-lymfom NOS. Metodickým základem bylo hodnocení morfologické, imunohistochemické ze vzorků fixovaných formalínem a zalitých do parafínu, využití klasifikačního schématu dle Hansové a spolupracovníků, detekce exprese Bcl-2, CD5, CD20, CD30 a NFκB v korelaci s klinickým nálezem (Ann Arbor stage, IPI, aa-IPI, PFS, OS), nálezem laboratorním a cytogenetickým (komplexní a simplexní karyotyp). Statistické zpracování zahrnovalo Cox regresivní analýzu a testy Mann-Whitney a Kruska-Wallis. Hodnoty doby do progrese onemocnění a celkového přežití byly stanoveny pomocí Kaplan-Meier analýzy. Při aplikaci klasifikátoru Hansové bylo rozpoznáno 11 případů (18,7 %), které nebylo možno zařadit jednoznačně k GCB/nonGCB-like podskupině. Všechny relabující případy vykazovaly negativní expresi CD10 a 28 případů bez detekovaného relapsu neslo pozitivní expresi znaku CD10. „Třetí" – GCB-like/nonGCB-like nezařaditelná podskupina měla křivku doby do progrese onemocnění velmi podobnou s křivkou nonGCB-like podskupiny, nicméně bylo zjištěno horší celkové přežívání. Statisticky nesignifikantně lepší odpověď na chemoterapii byla detekována u případů s pozitivní expresí Bcl-2 více než 30 %. Statisticky nesignifikantně lepší celkové přežití a delší dobu do progrese vykazovaly případy s proliferačním indexem více než 70 %. Studie zjistila jadernou expresi p50 v 17 případech (28,8 %) a v jednom případu jadernou expresi p65 (1,7 %), která může ukazovat na aktivaci signální dráhy NFκB. Statisticky nesignifikantní vztah byl zjištěn mezi expresí p50 a celkovým přežíváním/dobou do progrese onemocnění.

Klíčová slova: difuzní velkobuněčný B-lymfom – klasifikace – prognóza – imunohistochemie – NFκB signální dráha – formalínem fixovaná tkáň zalitá do parafínu.

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The experimental platform in hematooncology and malignant lymphoproliferations is still searching for new and more valid prognostic and predictive factors and appropriate sorting on clinical, morphological and molecular levels. Diffuse Large B-cell Lymfoma constitutes app. 30 – 40 % of nonHodgkin lymphomas (1). Median age is in the 7th decade (1). The potential prognostic and predictive factors in diffuse large B-cell lymfoma can be evaluated on clinical and histomorphologic grounds with the contribution of the immunoprofile and molecular profile (1,35). The neoplastic lymphoid cells of DLBCL are usually

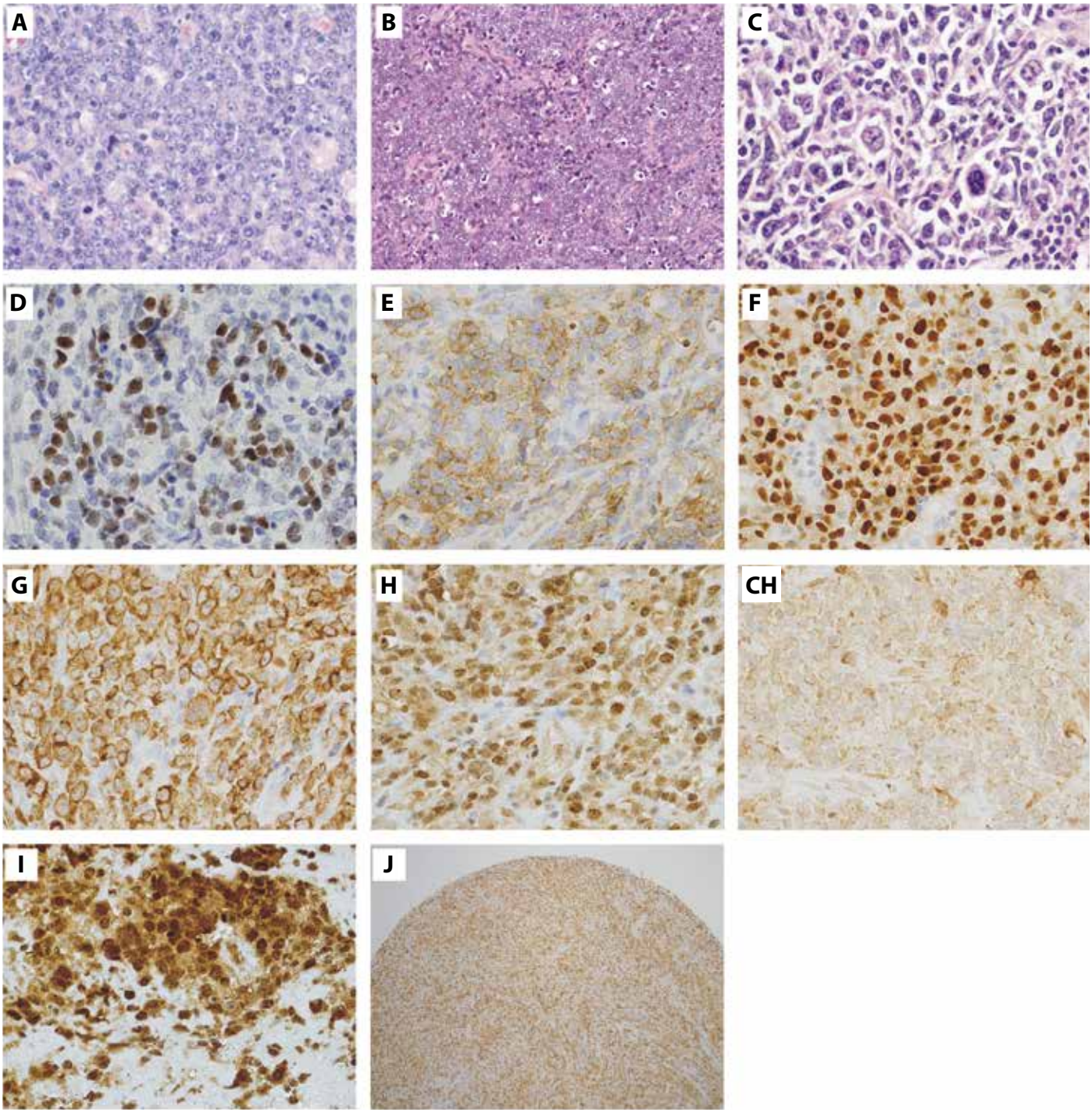


Fig. 1. Morphological variants of DLBCL NOS and expressions of immunohistochemically analysed determinants. A. Centroblastic DLBCL NOS (Giemsa stain, magnification 400x). **B.** Imunoblastic DLBCL NOS eith "starry-sky" pattern (HE, magnification 200x). **C.** Sarcomatoid variant of DLBCL NOS with HRS-like cells (HE, magnification 400x). **D.** Bcl-6 nuclear expression (magnification 400x). **E.** CD10 membranous expression (magnification 400x). **F.** MUM1/IRF4 nuclear expression (magnification 400x). **G.** Bcl-2 cytoplasmatic expression (magnification 400x). **H.** p50 nuclear expression (magnification 400x). **I.** p50 cytoplasmatic expression (magnification 400x). **J.** p65 nuclear expression (magnification 400x). **K.** Ki67 nuclear expression, tissue microarray (TMA) specimen (magnification 400x).

large with a nucleus twice the size of a reactive lymphocyte or a nucleus equal to or larger than that of reactive histiocytes (1). Neoplastic lymphoid cells may be medium sized but are different in nuclear and cytoplasmic features from the medium sized B-cell lymphomas (Burkitt´s lymphoma, B-cell lymphoblastic lymphoma). Nucleoli are almost already conspicuous, multiple (centroblastic type, Fig. 1A) or solitary (immunoblastic type, Fig. 1B) and nuclei may also show highly variable morphology HRS-like, bizzare, multinucleated (anaplastic type, Fig. 1C). Mitotic activity is commonly, easily identified and mitotic figures may be atypical.

The highly variable histology and cytology of DLBCL is a reflection that this lymphoma actually forms several different and yet incompletely distinguishable entities. Background reactive cells are also very variable and known cases of DLBCL rich in T-cell, histiocytes and eosinophils exist (13). DLBCLs express pan-B markers CD19, CD20, CD22, CD79a, PAX5 and may show variable positivity of these B-markers. Surface and/or cytoplasmic immunoglobulins can be demonstrated in app. 50 – 75 % of DLBCL cases (usually IgM) and cases with plasmocytoid differentiation are more commonly positive for cytoplasmic immunoglobulins (1). App.

10 % of DLBCLs express CD5, 30 % express CD10, 80 % express Bcl-6 (irrespective of BCL-6 rearrangement, Fig. 1D), 75 % express Bcl-2 (Fig. 1G), 10 % express CD30 (1,14). The proliferation fraction (Ki67, Fig. 1K) is usually more than 40 % up to 95 % (1,14).

Rearrangement of the BCL-2 gene occurs in 20 – 30 % of DLBCLs (1,14). Reciprocal chromosomal translocation involving the 3q27 region is shown up to 30 % of DLBCLs, BCL-6 rearrangement is detected in more than 30 % of DLBCLs and its presence correlates strongly with an extranodal involvement. BCL-2 and BCL-6 rearrangements are mutually exclusive in DLBCLs. The translocation of the protooncogen c-MYC is detected in app. 10 – 20 % of DLBCLs, and in app. 10 – 20 % of DLBCL cases the RB gene, tumor supresor gene, is inactivated. There is an extensive hunt (documented in literature) for prognostic markers to separate DLBCLs with a more favourable outcome from more aggressive tumours (15,43). The proliferation rate and EBV status are still being considered prognostic markers.

The potential prognostic and predictive factors in DLBCL can be evaluated on clinical and histomorphologic grounds with a contribution of an immunoprofile and molecular profile (1,35,44-46). Some studies used the immunohistochemically detected protein expression for the stratification of DLBCL (16-19,47). The most well-known is the "Hans classifier" where a combination of CD10, Bcl-6 and MUM1/IRF4 (Fig. 1E,1D,1F resp.) is used to sort DLBCLs into two groups GCB-like, nonGCB-like with app. 80% concordance with gene expression profiling (3). The new immunostaining classifier of DLBCL which is sorted into GCB-like, nonGCB-like and an unclassified subtype can be applied with app. 93% concordance with GEP (10) using GCET1, CD10, Bcl-6, MUM1/IRF4 and FOXP1 (10). Two major patterns of gene expression by a gene array technology have been proposed (2,21) dividing DLBCLs into prognostically significant subgroups: so called Germinal centre B-cell-like (GCB-like) and an Activated B-cell-like (ABC-like) DLBCLs. The expected 5-year overall survival (OS) in GCB-like and ABC-like DLBCL is app. 70 % and 39 % resp. (1). The first publication describing NFκB pathway is mentioned in 1986 (1) and comprises a family of transcription factors (RelA, RelB, c-Rel, p105, p100, p50, p52) with an important role in cell proliferation, antiapoptotic function and differentiation. The NFκB signalling pathway is activated by numerous stimuli, including bacteria and viruses and is referred to as a central mediator of an immune response and controls the expression of many inflammatory cytokines, chemokines, immune receptors and cell surface adhesion molecules (7,8,23-32). NFκB signaling pathway regulates the survival of normal and malignant B-cells by controlling the expression of cell death regulatory genes (7,8). The extrinsic apoptotic pathway is triggered by the engagement of the tumor necrosis factor (TNF) family death receptors (TNFR1, TNFR6/FAS/CD95) and the intrinsic apoptotic pathway is activated by the translocation of proapoptotic BCL2 family members to the mitochondria with subsequent release of cytochrome c (7,8,23-32). NFκB target genes enhance cell survival by modulating TNFα signaling, inhibiting FAS-mediated apoptosis and limiting the activity of proapoptotic BCL2 family members (7,8,23-32). The functional analysis in DLBCL´s cell lines with ABC-type signatures show high levels of NFκB activity and also the increased sensitivity to NFκB inhibition that specifically implicates the NFκB survival pathway in ABC-type of DLBCL. The constitutive activation of NFκB signaling pathway may contribute to the lymphomagenesis in DLBCL (8,33), expression of NFκB proteins can be identified by immunohistochemistry (Fig. 1H-1J) and the potential benefit from targeted anti-NFκB therapeutic approaches, e.g. bortezomib, rituximab is proposed (34,36).

MATERIALS AND METHODS

The retrospective and prospective designed project, patients with the diagnosis of particular non-Hodgkin B-lymphoproliferative disorder - DLBCL NOS has been included – all 59 patients with de novo DLBCL NOS (Tab. 2B, 4) and the median age was 64 years (Tab. 2A). The only criteria for the selection of patients were adequate formaline-fixed and paraffin-embedded material, the availability of all targeted clinical data, and offered and signed informed consent according to the Declaration of Helsinky. The number of affected lymph nodes (PET/CT) achieved n=16 with a median of 2 lymph nodes (Tab. 2A). The classification and subclassification of DLBCL NOS has been assessed according to the WHO classification scheme (1), staged according to Ann-Arbor scheme (Tab. 3) and the current clinical prognostic stratification according to IPI/aa IPI (Tab. 2A). We have also been monitoring the peripheral blood count, LDH, ß2-microglobulin serum levels and other laboratory evaluations and their results (Tab. 2A, B). The FFPE diagnostic tissue has been processed in the routine tissue sections (app. 5µm), placed on plus-slides and after the

Tab. 1. Primary antibodies.

Antibodies	Clone	Antigen retrieval	Dilution	Source
Anti-CD20	L26	MW	1:100	Dako
Anti-CD10	56C6	MW and methanol unblocking	1:40	Novocastra
Anti-CD30	Ber-H2	Water bath with EDTA and pH 9,0	1:100	Dako
Anti-Bcl-2	100 (124)	MW	1:10 (1:100)	Biogenex (Dako)
Anti-Bcl-6	PG-B6p	Water bath with pH 9,9 for 40min, or CSA kit for 20min and water bath with pH 9,9	1:20	Dako
Anti-MUM1/IRF4	MUM1p	MW	1:50	YbuxCytomation (Dako)
Anti-Ki67	MIB-1	MW	1:200	Dako
Anti-p50	Polyclonal	MW	1:400	Cell Signaling
Anti-p52	18D10	MW	1:50	Cell Signaling
Anti-p65	F-6	MW	1:400	Santa Cruz

MW – microwave oven, EDTA – ethylenediaminetetraacetic acid, pH – potential of Hydrogen

Tab. 2A. Data file descriptions.

	N	Mean	Median	Min	Max	25% quantile	75% quantile	SD
Age	59	59.8	64.0	26.0	84.0	47.0	73.0	16.5
No of positive LN	59	2.9	2.0	0.0	16.0	1.0	3.0	3.2
Max. diameter of LN in cm	51	8.9	7.0	1.8	25.4	4.0	13.0	6.1
Hb level	59	123.5	124.0	77.0	160.0	114.0	133.0	17.3
Leukocyte count	59	8.3	7.9	2.9	20.3	6.1	9.4	3.5
Trombocyte count	59	277.4	254.0	64.0	588.0	179.0	372.0	121.8
Lymphocyte count	59	1.8	1.4	0.3	8.5	0.9	2.2	1.5
ß2microglobulin level	51	3.9	3.1	1.3	15.3	2.1	5.4	2.7
s-TK level	58	28.4	13.9	2.0	100.0	7.3	31.3	31.5
Ca 125 level	49	66.5	33.0	3.5	595.5	13.8	67.5	102.4
IL-2 level	28	1.3	1.0	1.0	6.4	1.0	1.0	1.2
rIL-2 level	41	144.3	87.3	13.0	400.0	47.5	207.9	129.9
IPI	59	2.1	2.0	0.0	5.0	1.0	3.0	1.5
aa-IPI	38	1.1	1.0	0.0	3.0	0.0	2.0	1.0

LN - Lymph node, Hb - Hemoglobin, s-TK - Serum thimidine kinase, IL-2 - Interleukin 2, rIL-2 - Interleukine 2 receptor, IPI - International prognostic index, aa-IPI - Age adjusted international prognostic index

Tab. 2B. Data file descriptions.

		N	%
B symptoms	yes	25	42.4 %
	no	34	57.6 %
BM involvement	yes	5	8.6 %
	no	53	91.4 %
LDH	elevated	28	47.5 %
	normal	31	52.5 %
Cytogenetics	negative	22	81.5 %
	positive	5	18.5 %
CHT	(R)PACEBO	9	15.3 %
	(R)CHOP	30	50.9 %
	others	19	32.2 %
PS	0	12	20.3 %
	1	38	64.4 %
	2	5	8.5 %
	3	4	6.8 %

BM - Bone marrow, LDH – Lactate dehydrogenase, CHT - Chemotherapy, PS - Performance status

Tab. 3. Ann Arbor Stage.

Stage	I	9	15.3 %
	II	16	27.1 %
	III	10	16.9 %
	IV	24	40.7 %

Tab. 4. Gender.

Gender	F	32	54.2 %
	M	27	45.8 %

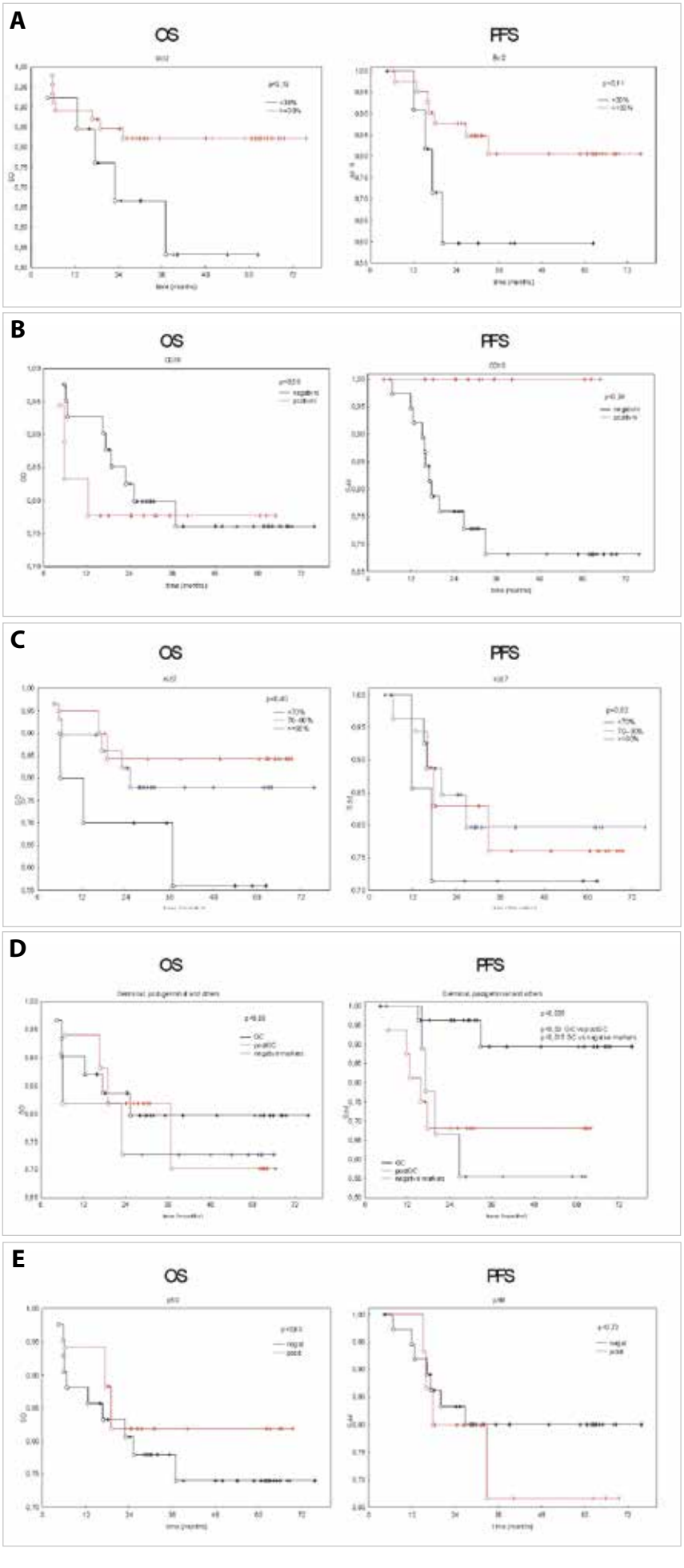
F - Female, M - Male

antigen retrieval the indirect immunohistochemistry has been processed with the application of commercially available primary antibodies for the particular detected proteins (standard protocol according to the manufacturer's´s manual) in optimalised dilution (Tab. 1). The visualisation of secondary antibody has been performed with the usage of the standard avidin-biotin (ABC) method. The immunohistochemically detected positivity and negativity (positive and negative protein expression) of analysed determinants/proteins (CD10, Bcl-6, MUM1/IRF4) has been designed according to the Hans classifier (1), positivity of Bcl-2 was correlated on 5%, 10% a 30% level, positivity of Ki67 (proliferation index) was correlated at levels of < 70% (including 40% discriminatory level for DLBCL diagnosis), 70 – 90% and > 90%. Any positivity of p50, p52, p65 (nuclear expression) has been recorded in % and subsequently correlated.

All statistical analyses were performed with the application STATISTICA 10 software (Statsoft, Inc.). Value $p < 0.05$ was thought to be statistically significant. The prognostic value(s) of the immunohistochemically detected and aforementioned factors have been analysed with Cox regressive analysis, Mann-Whitney and Kruska-Wallis test. The interval of PFS and OS has been assessed according to Kaplan-Meier analysis.

RESULTS

Our study includes 17 cases (28.8 %) of DLBCL NOS that showed nuclear positivity of p50 expression, only one case indicated a nuclear positivity of p65 expression (1.7 %) and no positivity of p52 expression has been ascertained (Tab. 6). According to the design of the Hans classifier we have selected 31 cases (52.5 %) of the GCB-like subgroup of DLBCL, 17 cases (28,8 %) of the nonGCB-like subgroup of DLBCL and in 11 cases (18.7 %) we have not been able to sort them precisely into any of the aforementioned subgroups according to the level of positive coexpressions (CD10, MUM1/IRF4 near 30 % or higher between 30 % and 80 %). The most common stage of the Ann Arbor staging system was stage IV (40.7 %). The detected morphology of DLBCL (centroblastic, immunoblastic and anaplastic) was related to



proliferation index Ki67 and expression of CD30 without any significant results. The expression of Bcl-2, Bcl-6, CD10, MUM1/IRF4, CD30, Ki67, p50, p65 (Tab. 6) and subgrouping into GCB-like, nonGCB-like and a "third" group have been correlated to OS and PFS. Statistically significant differences in PFS have been recorded in CD10 expression (Fig. 2), GCB-like, and nonGCB-like subgroups of DLBCL cases (Fig. 2). All relapsing cases (n = 11) bear negative expression of CD10 and 28 cases without any detected relapse of DLBCL showed positive expression of CD10 (Tab. 7).

The majority of GCB-like cases (n = 26) performed no signs of the disease progression and only 11 cases of the nonGCB-like subgroup exhibited no progression and consequently progression in the nonGCB-like subgroup has been more frequent in comparison with the GCB-like subgroup (n = 5 versus n = 2 resp., Tab. 8). The "third" - GCB-like/nonGCB-like unsortable subgroup (18.7 %) shared very similar courses (a similar curve) of PFS with the nonGCB-like subgroup and a worse clinical course of OS (Fig. 2). Nearly statistically significantly better responses or detected trends for a better response to chemotherapy (CR) were shown by cases (n = 30) with positive Bcl-2 expression of more than 30 % and only in 8 cases has PR been detected, while PD has been detected in 4 cases PD (Tab. 9). Statistically nonsignificantly better OS and PFS was shown by cases with an upper level of proliferation index Ki67 (more than 70 %) compared to a lower proliferative index (under 70 %) of DLBCL NOS cases (Fig. 2). A statistically non-significant relationship of p50 expression with OS and PFS was presented (Fig. 2). A higher level of leukocytosis has been found statistically significant in relation to Bcl-6 and p50 expressions, a higher level of lymphocytosis has been statistically significantly correlated with positive expression of CD10 and negative expression of CD30. Significantly higher levels of s-TK have been detected in DLBCL NOS cases with Bcl-2 positivity more than 5 %. Cases with negative expression of MUM1/IRF4 showed a statistically significantly higher ß2microglobulin level.

DISCUSSION

From the beginnings of malignant hematolymphoid classification(s) development, it has been known that the separately formed/classified neoplastic lymphoproliferative disorders and also DLBCL (including DLBCL NOS as well) are not uniform entities and their heterogeneity

Fig. 2. OS and PFS according to observed determinants. A. OS and PFS acc. to Bcl2 expression. **B.** OS and PFS acc. to CD10 expression. **C.** OS and PFS acc. to proliferation index Ki67. **D.** OS and PFS acc. to GCB, nonGCB-like and "third" subgroup of DLBCL. **E.** OS and PFS acc. to p50 expression.

Tab. 5. DLBCL NOS sorting acc. to Hans classifier.

	GCB-like	31	52.5 %
	NonGCB-like	17	28.8 %
	Unsorted	11	18.7 %

GCB-like - Germinal centre B-cell like, NonGCB-like – Non-germinal centre B-cell like

Tab. 6. Immunohistochemically detected expressions.

		n	%
Bcl-2	> = 5 %	52	88.1 %
	< 5 %	7	11.9 %
Bcl-2	> = 10 %	50	84.8 %
	< 10 %	9	15.3 %
Bcl-2	>= 30 %	46	77.9 %
	< 30 %	13	22.0 %
Bcl-6	negative	23	38.9 %
	positive	36	61.0 %
CD10	negative	41	69.5 %
	positive	18	30.5 %
CD30	negative	43	72.9 %
	positive	16	27.1 %
MUM1	negative	34	57.6 %
	positive	25	42.4 %
Ki67	< 70 %	10	16.9 %
	70 – 90 %	20	33.9 %
	>= 90 %	29	49.2 %
p65	negative	58	98.3 %
	positive	1	1.7 %
p50	negative	42	71.2 %
	positive	17	28.8 %

is mirrored in all currently-used diagnostic levels - clinical, laboratory, morphological and molecular ones as well as the unique timeline of evolution for particular malignant lymphoproliferative cases should be taken into account. Prognostic and predictive stratification of DLBCL NOS with regard to gene expression analysis (GEP) is very well known and accepted (1), but is not widely applied in a routine diagnostic due to its eminent price and therefore new appropriate methodological options are being searched for.

The immunohistochemistry is one of the familiar and broadly available methodological opportunities that has already been used with app. 80% concordance between the Hans classifier (3) and GEP and app. 93% concordance among the Choi classifier (10), and in the analyzed specimen(s) we can also establish the topography of the positive expression (neoplastic lymphoid cells versus non-neoplastic milieu). The immunohistochemical DLBCL NOS subgrouping plays an important biological role not only in GCB and nonGCB-like sorting but also single positive expression of CD5 is an adverse prognostic factor (35) and single positive expression of CD23 is a favorable factor (35) of *de novo* DLBCL´s. The subgroup of DLBCL NOS with a lower coexpression level of CD10 and MUM1/IRF4 (near 30 %) or with a higher coexpression level (30 % to 80 %) is presumably of late GCB-like or early nonGCB-like origin with downregulation of both CD10 and Bcl-6 and upregulation of MUM1/IRF4.

Tab. 7. CD10 expression and relapsing or progressive disease.

		CD10		
	p<0.025	negative	positive	total
Relapse/progression	yes	11	0	11
	no	28	15	43
	total	39	15	54

Tab. 8. GCB/nonGCB-like/"third" subgroup and relapsing or progressive disease.

	p<0.04	GCB	nonGCB	"third"	total
Relapse/ progression	yes	2	5	4	11
	no	26	11	6	43
	total	28	16	10	54

Tab. 9. Relationship Bcl-2 expression and CHT response.

		Bcl-2 expression		
	p<0.07	< 30 %	>= 30 %	total
CHT response	SD	1	0	1
	CR	6	30	36
	PR	5	8	13
	PD	0	4	4
	total	12	42	54

SD – stable disease, CR – complete remission, PR – partial remission, PD – progressive disease

Another adverse prognostic factor - the constitutive activation of NFκB signaling pathway is proposed to be related to the ABC-type of DLBCL (11), clinically more aggressive type of DLBCL and the analysis of NFκB constitutive activation status can be also suggested for targeted therapy in positive cases. Our study showed that p50 and p65 positive cases of DLBCL NOS did not bear any statistically significant correlation with all observed determinants but in spite of this finding the NFκB activation was detected and concerned patients could profit from this therapeutic target. Statistically nonsignificantly better OS and PFS were shown in DLBCL NOS cases with higher proliferation (proliferation index Ki67 more than 70 %) in comparison with a lower proliferative index (under 70 %) - presumably because of a better therapy response in highly proliferative cases of DLBCL NOS but the native course of DLBCL NOS with higher neoplastic proliferation is expected to be related to adverse progress of the disease and in the same manner the above-mentioned adverse prognostic factor - NFκB constitutive activation – could be a favourable predictive factor due to the applied targeted approach (probably not only) in DLBCL NOS. Therefore the immunohistochemical DLBCL NOS profile including NFκB activation analysis could be proposed to refine the subgrouping of DLBCL (or not only DLBCL NOS), positive expression of NFκB could also be intended for the targeted therapy (e.g. an application of proteasome complex inhibitor – bortezomib, resveratrol, celastrol, dexamethason, etc.).

CONCLUSIONS AND NEW PERSPECTIVES

DLBCLs (and also other malignant lymphoproliferative disorders in general) show a huge and still evolving heterogeneity on a clinical, morphological and molecular diagnostic level and a tight

and clearcut cooperative hematological team including clinicians, hematopathologists, molecular biologists and statisticians is a real benefit for a correct diagnosis with a potential influence on new kinds of targeted and tailored therapeutic approaches and also on new insight(s) into both the experimental algorithms and algorithms of translation medicine closer to the real patient(s).

We can also mention new possibilities in lymphoma immunoprofiling. The cell surface capture technology (CSC) is a mass spectrometry-based method that can indentify cell surface glycoproteins including clusters of differentiation (CD) proteins (37). CSC can be a part of a systematic and quantitative analysis of differentially expressed cell surface proteins (37).

The idiotypic vaccination for B-cell lymphomas is another powerful and more harmless new therapeutic approach. B-cell lymphoproliferative diseases bear proven immunogenicity that can be used in the new immunotherapeutic strategy era (anti-lymphoma vaccines) due to tumor-specific immune response and even lymphoma remission on the molecular level currently described in follicular lymphoma (38). On the other hand the development of anti-lymphoma vaccines, individualized idiotypic vaccines, is still time-consuming and a very expensive complex process (38).

The molecular basis of lymphoma dissemination is the next challenge for learning of lymphoma biology. A number of clinical observations suggest that conserved homing programs mediate the dissemination of non-Hodgkin lymphoma, e.g. B-chronic lymphocytic leukemia/small lymphocytic lymphoma and mantle cell lymphoma usually show systemic dissemination at presentation whereas NHLs related to lymphocytes undergoing active proliferation and differentiation such as DLBCL and BL are often initially localized (39). The lymphoma microenvironment e.g. the decreased immunosurveillance may play a role in DLBCL pathogenesis due to the impaired anti-neoplastic and anti-viral immunologic reaction. A very good experimental model for the imunocompromised state related lymphoma is AIDS-related DLBCL (AR-DLBCL). The AR-DLBCL compared to the sporadic DLBCL shows high level of angiogenesis, increased proliferation, reduced count of CD4+ and FOXP3+ subsets of T-helper cells, increased activated cytotoxic T-cells and few tumor-associated macrophages (40). A very robust R-IPI independent prognosticator is the status of circulating host immunity (reflecting intratumoral immune microenvironment) and flow cytometry performed on a fresh diagnostic lymphoma tissue (DLBCL) revealed high and low risk groups according to percentage of CD4+ infiltrating T-helper cells, under 23 % and greater than or equal to 23 % of CD4+ T-cells resp. (41,42).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

Abbreviations: aa-IPI – Age adjusted International prognostic index, Ab´s – Antibodies, ABC – Avidin-biotin, ABC-like – Activated B-cell like, AR-DLBCL – AIDS related DLBCL, BCL-2 – B-cell CLL/lymphoma 2, BCL-6 – B-cell CLL/lymphoma 6, BM – bone marrow, B-NHL – B-cell non-Hodgkin lymphoma, CD – Cluster of differentiation (cluster of designation, classification determinant), CD10 – Cluster of differentiation 10, CD20 – Cluster of differentiation 20, CD30 – Cluster of differentiation 30, CD5 – Cluster of differentiation 5, c-Myc – Proto-oncogen, myelocytomatosis oncogene, CR – complete remission, CSC – cell surface capture technology, DLBCL – Diffuse large B-cell lymphoma, EBV – Epstein-Barr virus, EDTA – Ethylenediaminetetraacetic acid, FFPE – Formaline-fixed, paraffin-embeded, GC – Germinal center, GCB – Germinal center B-cell, GCB-like – Germinal centre B-cell like, GEP – Gene expression profile, HRS – Hodgkin/Reedberg-Sternberg cell, CHOP – Chemoterapy regimen - Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine), Prednisone/Prednisolone, CHT – Chemotherapy, IgM – Immunoglobulin heavy-chain M, IHC – Immunohistochemistry, IKK – Inhibitor κB kinase, IPI – International prognostic index, IκB – Inhibitor κB, IκBα – Inhibitor κBα, LDH – lactate dehydrogenase, MW – microwave oven, NFκB – Nuclear factor κ light-chain enhancer of activated B cells, nonGCB – Nongerminal center B-cell, NOS – Not otherwise specified, OS – Overall survival, PAX5 – Paired box 5 (Family of transcription factors, B-cell lineage specific activator protein - BSAP), PD – progressive dinase, PFS – Progression free survival, pH – potential of hydrogen (pondus hydrogenia), PR – Partial remission, PS – perfomance status, SD – Standard deviation (contextual), SD – Stable disease (contextual), TMA – Tissue microarray, TNF – Tumor necrosis factor, WHO – World Health Organisation

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