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Distinguishing of primary mediastinal B-cell lymphoma and diffuse large B-cell lymphoma using real-time quantitative polymerase chain reaction

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Primary mediastinal B-cell lymphoma (PMBL) seems to be reliably distinguished from diffuse large B-cell lymphoma (DLBCL) with microarray technology. We measured expression of *Fcer2, Pdl2* and *Blk* genes using real-time quantitative polymerase chain reaction (RTqPCR) on formalin fixed, paraffin embedded material (FFPE) and suggested a formula to discriminate PMBL from DLBCL. For 39/82 included patients the diagnosis of PMBL was expected clinico-pathologically. Diagnosis of 10/39 and 2/43 of clinically considered PMBLs and DLBCLs, respectively, was not genetically confirmed. Compared to confirmed PMBLs, unconfirmed ones showed clinical features similar to DLBCLs, e.g. spleen infiltration (p=0,028) and decreased invasiveness in pericardium (p=0,045). They tended to have more common infradiaphragmatic involvement, less often tumor sclerosis or fluidothorax. There were no immunohistochemical differences between genetically confirmed and unconfirmed PMBLs. New approach of distinguishing PMBL and DLBCL is presented. It is based on expression of three genes in routinely available FFPE material using RTqPCR.

Key words.; Gene expression; primary mediastinal B-cell lymphoma,diffuse large B-cell lymphoma; formalin fixed, paraffin embedded tissue ; real-time quantitative polymerase chain reaction

Primary mediastinal B-cell lymphoma (PMBL) is a subset of diffuse large B-cell lymphoma (DLBCL) accounting for 2-5% of patient with non-Hodgkin lymphoma (NHL) [1–4]. It typically affects young women [1, 5–7]. Bulky mediastinal masses, local invasiveness in the chest and elevated lactate dehydrogenase (LDH) level are common at the initial presentation [1, 2, 5–8]. Distant spread including bone marrow and cerebrospinal fluid often appears at the time of relapse [1, 2, 5]. PMBL was found to be genetically different from DLBCL using gene expression profiling [5]. However, there are no histological features reliably distinguishing these two entities and PMBL thus remains a clinico-pathological diagnosis only [8, 9].

Morphologically, PMBL is characterized by diffuse growth of medium to large B-cells associated with sclerosis of different degree. Focal nodularity, extensive necrosis, vascular invasion, thymic remnants and Reed-Sternberg-like features can be seen as well (2, WHO). PMBL typically expresses B-cell antigens (CD19, CD20, CD22) but often lacks surface immunoglobulin (Ig) despite expression of the Ig co-receptor CD79a. CD30, STAT1, TRAF1, MAL, FIG1, CD58 and CD54 protein staining is often but not regularly positive. Distinct chromosomal gains/amplifications and losses have been observed including consistent gains in chromosomes 9p and 2p [6, 10–12].

Two extensive studies of gene expression profiling [5, 9] confirmed NF-κB pathway activation [9, 13, 14], low expression levels of multiple B-cell signaling components [9] and genetic features shared with classical Hodgkin lymphoma (cHL) [5, 9]. 24% of patients for whom the diagnosis of PMBL was clinically considered were not assigned as PMBL using microarray technology. These discordant patients tended to have worse clinical outcome [5]. The goal of our study was to help to distinguish PMBL from DLBCL using limited number of genes, real-time quantitative polymerase chain reaction (RTqPCR) and formalin fixed, paraffin embedded tissue (FFPE).

Materials and methods

Patients and samples. 82 patients with DLBCL were included in the study. The DLBCL diagnosis was confirmed by two independent expert hematopathologists. Diagnosis of PMBL was done based on histopathology and clinical criteria (predominant mediastinal involvement over 7cm) in 39/82 patients. These patients were designated as „considered PMBLs" and were diagnosed de novo. Other 43 patients were designated as "considered DLBCLs". In this group, samples of eight patients were obtained at the time of disease progression and they were excluded from survival analysis. Patients were treated with antracycline-based regimens. In two patients therapy was not initiated. 72/82 patients received rituximab. The study was reviewed by the Ethics committee and was performed in accordance with the Helsinki Declaration.

Genes differentially expressed in PMBL and DLBCL. Genes were chosen based on published microarray data. The human leukocyte differentiation antigen (*Fcer2*) is a key molecule for B-cell activation and growth and is overexpressed in PMBL [5]. Programmed cell death 1 ligand 2 (*Pdl2*) encodes a regulator of T-cell activaton and is highly expressed in PMBL [5]. B-lymphoid tyrosine kinase (*Blk*) is a member of BCR signalling cascade with decreased expression in PMBL [9].

RNA isolation from fresh leukocytes. 20 ml of blood were obtained from 40 volunteers. RNA isolation from fresh leukocytes was processed following the protocol of Chromcinsky et al. (15) and stored at -80°C. The RNA quality was controlled on agarose gel. RNA from all samples was mixed and used as a calibrator in RTqPCR.

RNA isolation from FFPE. RNA was successfully isolated from 99% of initially obtained paraffin blocks. Paraffin blocks were cut 4 x 10 μm. RNA was extracted using High Pure RNA Paraffin Kit (Roche, Basel, Switzerland) based on glass fiber filter isolation under a specific salt condition. Samples were handled according to the manufacturer's instructions. RNA was spectrophotometrically evaluated.

Reverse transcription and RTqPCR. Reverse transcription was processed using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, Ontario, Canada) according to the manufacturer's instructions. Real-time quantitative PCR was performed using the Stratagene 3000Xp cycler and Taqman primer and probe system provided by Applied Biosystems (AB; Foster City, CA, USA). The length of the amplicons in RTqPCR experiments was within the range of 60 – 105bp. Expressions levels of the following genes were measured: *Beta-2-microglobulin* (*B2m*) Hs 00187842-m1, *Ribosomal protein P0* (*Rplp0*) Hs 99999902-m1, *CD23 receptor/Fc fragment of IgE* (*Fcer2*) Hs 00233627-m1, *Programmed cell death 1 ligand 2* (*Pdl2*) Hs 00228839-m1, *B lymphoid tyrosine kinase* (*Blk*) Hs 01017457-m1. RTqPCR for each AB probe was run in 20 μl reactions in duplicates or triplicates, using 10 μl of Taqman Universal PCR Master Mix and 1 μl of probe and primer mix according to the manufacturer's instructions. 500 μg and 100 μg of cDNA template from FFPE and fresh leucocytes were used for a single reaction, respectively. The temperature profile consisted of an initial step of 95°C for 10 min and 40 cycles of 95°C for 15 sec and 60°C for 1 min. In accordance to the latest recommendations, mRNA level of each gene of interest was normalized to the geometric mean of Ct values of two housekeeping genes (*B2m* and *Rplp0*). For calibration we used pooled leukocyte RNA from 40 volunteers, obtaining $\Delta\Delta C_T$ values for each gene in each sample. Intraassay variability was 0,7%, interassay variability 5%.

Testing and validation sets. 32 patients were included in the "testing" set, e.g. 11 and 21 patients with PMBL and DLBCL diagnosis, respectively. As mentioned above, diagnosis of PMBL was done based on histopathology and clinical criteria in 39 patients. To minimize the risk that also clinico-pathologically misdiagnosed PMBL patients will be included in the testing set, only PMBL patients with no extrathoracic involvement were included.

The "validation" set included 50 patients, for 28 of them the diagnosis of PMBL was considered.

Expression of *Fcer2, Pdl2* and *Blk* genes was examined in the testing set. Discrimination function was generated. The median values of expression of each gene acquired with $\Delta\Delta C_T$ method were compared in each group (PMBL and DLBCL). Then, a constant corresponding to a discriminating importance between two groups was given to each gene and a formula was generated.

Immunohistochemistry. Immunohistochemical staining using standard methods was performed on FFPE slides. Heat induced epitope retrieval was used for all antibody protocols, in case of BCL6 with use of high pH solution. This panel of antibodies was used: CD20 (DAKO, dilution 1:300), CD30 (DAKO, dilution 1:30), CD5 (Biogenex, dilution 1:30), CD23 (Neomarkers, dilution 1:100), (DAKO, dilution 1:100), CD10 (Novocastra, dilution 1:80), BCL6 (DAKO, dilution 1:15), MUM1 (DAKO, dilution 1:100). Samples were evaluated according to the algorithm published by Hans et al. [16] discerning between GC-like phenotype (CD10+BCL6+/-MUM1+/- or CD10-BCL6+MUM1-) and nonGC-like phenotype (CD10-BCL6+/-MUM1+ or CD10-BCL6-MUM1-).

Statistics. Gene expression in PMBL/DLBCL groups, association of gene expression and organ involvement and age comparison in different groups were analyzed using Wilcoxon test. The predictor formula was generated with canonical discriminant function. Progression free survival (PFS) was evaluated using Kaplan-Meier analysis and log-rank test. Crosstabs were used to compare organ involvement in PMBL and DLBCL groups. Kruskal-Wallis test was used to compare initial clinical stage and international prognostic index (IPI) in PMBL/DLBCL groups. SPSS software was used.

Results

Gene expression in the testing set and the predictor. In the testing set, *Fcer2* and *Pdl2* genes were higher expressed in

the PMBL group (p=0,002 for both genes), *Blk* was higher expressed in the DLBCL group (p=0,042). Based on these results, discriminant function was used to suggest a formula: F = + 0,029 * *Fcer2* + 0,006 * *Pdl2* – 0,012 * *Blk* – 0,168. This formula was termed the "predictor". If the value of the sample was negative, the sample was assigned to the "predicted DLBCL group", a sample of a positive value to the "predicted PMBL group".

Three patients in the testing set did not correspond to the expected expression of given genes and fell into the other predicted group. Two of them belonged to the considered DLBCL group and the third was of initial considered PMBL diagnosis.

*Validation Set***.** 50 samples were tested in the validation set. Out of them, 28 patients belonged to the considered PMBL group. With the predictor formula, 9/28 of expected PMBL patients fell into the predicted DLBCL group. All the 22 patients from considered DLBCL group in the validation set were assigned to the predicted DLBCL group.

Gene expression in the whole group. In summary, 82 patients were tested with the formula. In the whole group, 10/39 (26%) and 2/43 (5%) of considered PMBLs and DLBCLs, respectively, did not correspond to the initially considered diagnosis (Table 1). Patients for whom the considered diagnosis was confirmed by the predictor were assigned as "concordant", the others as "discordant". The distribution of discordant patients across the testing and validation sets is shown in Table 2.

Expression of *Fcer2* was positively associated with tumor sclerosis (p=0,005) and bulky disease (p<0,001) and negatively with an infradiaphragmatic involvement (p=0,009). Expression of *Pdl2* gene was associated positively with tumor sclerosis (p=0,001) and with bulky disease (p<0,001). Expression of none of the genes was associated with involvement of lung, pleura or spleen.

Table 1: Patients divided according to the clinical diagnosis and to the predictor.

	Predicted PMBL	Predicted DL-BCL	total
Considered PMBL	29	10	39
Considered DLBCL	2	41	43
Total	31	51	82

Table 2. Discordant patients in testing and validation sets.

	Discordant PMBL	Discordant DLBCL
Testing set (%)	9	10
Validation set (%)	32	0
Total (%)	26	5

Clinical Features. Clinical data were evaluated separately in the "considered" and "predicted" groups. Results are summarized in Table 3.

In both groups, PMBL patients were significantly younger and women were predominant. Infradiaphragmatic involvement was significantly more common in the DLBCL group, PMBL patients had more often elevated LDH levels, pericardium infiltration, fluidothorax and tumor sclerosis and none of them in either group had any bone marrow involvement at the time of diagnosis. 11 patients manifested superior vena cava syndrome (SVCS). All of them were clinico-pathologically considered PMBLs, two of them belonged to the discordant genetic group. There were no significant differences between the PMBL and

Table 3. Comparison of clinical data of PMBL and DLBCL patients in considered and predicted groups.

	Considered diagnosis			Predicted diagnosis		
	PMBL (n=39)	DLBCL (n=43)	p value	PMBL (n=31)	DLBCL (n=51)	p value
Median age (years)	33	60	*p*<0,001*	30	59	*p*<0,001*
Women (%)	74	51	*p*=0,031*	77	53	*p*=0,027*
Infradiaphragmatic (%)	33	67	*p*=0,004*	30	65	*p*=0,002*
Lung (%)	18	7	*p=0,184*	19	8	*p=0,131*
Spleen (%)	15	19	*p=0,699*	6	24	*p=0,068*
Pleura (%)	10	2	*p=0,19*	13	2	*p=0,068*
Pericardium (%)	23	0	*p*=0,001*	29	0	*p*<0,001*
Fluidothorax (%)	51	10	*p*<0,001*	52	16	*p*=0,001*
Chest wall (%)	10	0	*p*=0,049*	10	2	*p=0,154*
SVCS (%)	28	0	*p*<0,001*	30	4	*p*=0,002*
Elevated LDH (%)	92	61	*p*=0,001*	94	65	*p*=0,003*
Bone marrow (%)	0	18	*p*=0,031*	0	15	*p=0,078*
Tumor sclerosis (%)	77	33	*p*<0,001*	81	37	*p*<0,001*

Statistical significant data are indicated with an asterisk. The number of patients (n) in each group is given in brackets. These data was missing: LDH level with three patients, bone marrow involvement with six patients and lung, pleura, pericardium, fluidothorax and chest wall infiltration with one patient. The percentage sign in the left column indicates for percentage of patients with the given sex and involvement.

Table 4. Comparison of clinical data of discordant PMBL versus concordant PMBL patients and concordant DLBCL ones.

	Dis. versus con. PMBLs			Dis. PMBLs versus con.DLBCLs		
	Dis. PMBL (n=10)	Con. PMBL (n=29)	p value	Dis. PMBL (n=10)	Con. DLBCL (n=40)	p value
Mean age (years)	46	30	*p*=0,037*	46	60	*p=0,039**
Women (%)	60	79	*p=0,228*	60	50	*p=0,571*
Infradiaphrag.(%)	50	28	*p=0,195*	50	70	*p=0,232*
Lung (%)	20	17	*p=0,845*	20	5	*p=0,180*
Spleen (%)	40	7	*p*=0,028*	40	20	*p=0,185*
Pleura (%)	0	14	*p=0,556*	0	3	*p=1,000*
Pericardium (%)	0	32	*p*=0,045*	0	0	*NA*
Fluidothorax (%)	40	62	*p=0,408*	40	10	*p*=0,044*
Chest wall (%)	1	10	*p=1,000*	1	0	*p=0,204*
SVCS (%)	20	32	*p=0,504*	20	0	*p*=0,037*
Elevated LDH (%)	67	100	**p=0,01*	67	66	*p=0,96*
Bone marrow (%)	0	0	*NA*	0	18	*p=0,156*
T. sclerosis (%)	60	83	*p=0,141*	60	33	*p=0,109*

Statistically significant data is indicated with an asterisk. Missing data is explained in Table 3. Dis., discordant; con., concordant; infradiaphrag., infradiaphragmatic involvement; T. sclerosis, tumor sclerosis.

DLBCL groups in initial staging and international prognostic index (IPI), neither in considered nor in predicted set. A certain degree of sclerotic features was observed in 77% and 81% of considered and predicted PMBLs, respectively. In considered and predicted DLBCLs it was 33% and 37%, respectively.

Discordant Patients. 26% and 5% of considered PMBLs and DLBCLs, respectively, did not correspond to the clinically considered diagnosis when gene expression was taken into consideration. Clinical data of concordant and discordant patients is compared in Table 4.

Altogether, six patients with mediastinal bulk had infiltration of spleen and four of them belonged to the discordant group (p=0,028). None of the discordant patients showed pericardium infiltration (p=0,045). Three considered PMBL patients had initially no elevated LDH and all of them were discordant (p=0,01). Compared to concordant PMBL cases, discordant patients were older (p=0,037). They tended to have more common infradiaphragmatic involvement, they had less often tumor sclerosis, fluidothorax, SVCS or chest wall involvement and none of them had pleura involvement.

Compared to concordant DLBCL patients, discordant PMBL ones were younger (p=0,039) and presented more often fluidothorax (p=0,004) and SVCV (p=0,037). Other clinical differences were not statistically significant.

2/43 patients with initially considered DLBCL diagnoses fell into the predicted PMBL group. Both the patients were in the first CR with follow-up 46 and 40 month, respectively.

PFS of PMBL and DLBCL patients was compared in both, considered and predicted groups. In both groups, PMBL patients tended to have superior outcome. In the considered group the result was not statistically significant (p=0,179), in the predicted one marginally significant (p=0,084).

Immunohistochemistry. NonGC-like phenotype was found in 53% (20/38) of concordant DLBCLs, in 61% (17/28) of concordant PMBL (p=0,618) and in 90% (9/10) of discordant PMBL group (p=0,124). One discordant DLBCL patient was GC-like, the other one nonGC-like. CD23 positive staining was found in 8% (3/39) of concordant DLBCLs, in 72% (21/29) of concordant PMBLs (p<0,001) and 40% (4/10) of discordant PMBLs (p=0,124). One discordant DLBCL patient was CD23+, the other one was CD23-. CD30 staining was positive in 15% (6/41) of concordant DLBCLs, in 64% (18/28) of concordant PMBLs (p<0,001) and in 80% (8/10) of discordant PMBLs (p=0,453). One discordant DLBCL patient was CD30+, the other one was CD30-. In the discordant PMBL group, two patients carried nonGC-like/CD23-/CD30- phenotype, four were nonGC-like/CD23-/CD30+, three were nonGC-like/CD23+/CD30+ and one was GC-like/CD23+/CD30+.

Discussion

Our study is focused on molecular distinguishing of DLBCL and PMBL. We analyzed a group of 82 clinico-pathologically classified DLBCL and PMBL patients. Based on published microarray data we chose *Fcer2, Pdl2* and *Blk* genes as potential classifiers between the mentioned groups. RTqPCR was run in testing set of 32 patients and the results were used to complete a mathematical formula called predictor. With the predictor all 82 patients were finally reclassified to genetic DLBCL and PMBL subgroups. These genetic groups were partially overlapping with the clinico-pathological ones. Thus, discordant PMBL, discordant DLBCL, concordant PMBL and concordant DLBCL groups were finally analyzed. The group of discordant PMBLs showed some clinical features similar to that of concordant DLBCL ones.

There are no immunohistochemistry markers exclusively typical for PMBL. We were however interested in the immunohistochemical staining of typical PMBL markers as well as in the immunohistochemical GC/nonGC-like classification in the whole group of patients. As for PMBL markers, we used usual available and routinely examined markers CD23 and CD30. Immunohistochemistry confirmed higher incidence of CD23 and CD30 positive staining in clinico-pathologically considered PMBL tumors. Discordant PMBL patients tended to be more often CD23 negative. However, negative immunohistochemical staining of CD23/CD30 did not change the clinico-pathologically considered diagnosis of PMBL.

The cell of origin of PMBL is most probably a thymic medulla B-cell but also a similarity to GC-like or nonGC-like DLBCL is discussed [17]. On gene expression level, PMBL was more similar to GC phenotype in the work of Rosenwald et al. In another work the uncommon presence of t [14; 18] translocation resembled rather the nonGC group [18]. In our study, considered PMBLs tended to be more often nonGC-like and this fact probably influenced the high rate of nonGC-like cases in the discordant PMBL group (90%). Taken together, there was no GC/nonGC-like/CD23/CD30 immunophenotype that would uniformly characterize the discordant PMBL group.

Particular attention was given to presence of tumor sclerosis. In some series, sclerosis has been defined as a diagnostic criterion of PMBL, but its variability in different areas of the same tumor makes this rather impractical [2]. Our data further confirms that sclerosis can be seen in up to 33% of DLBCLs from other localizations but not in 100% of PMBL cases.

39 patients were initially designated to the considered PMBL group. This diagnosis was confirmed by our predictor in 29 patients (74%). Of interest, further 2/43 of initially considered DLBCL patients (5%) were genetically predicted as PMBL. In the observation of Rosenwald et al [5], 76% of patients for whom the diagnosis of PMBL was clinically considered were classified as PMBL by gene expression. Further, 5% of clinically expected DLBCLs showed PMBL gene profiling in their validation set. Higher discordant rate in PMBLs in our validation set is attributed to the fact that the PMBL patients in the testing set were strictly chosen as patients with limited intrathoracic disease (Table 2).

Clinical features of PMBL and DLBCL groups judged separately by clinical diagnosis and by predictor were as expected in comparison with other authors [1, 2, 5–8]. In both the groups, PMBL patients were younger, in particular women, had more often elevated LDH levels and no bone marrow involvement at the time of diagnosis. 33% and 30% of "considered" and "predicted" PMBL patients, respectively, had also infradiphragmatic involvement. In the group of "considered" and "predicted" DLBCLs it was 67% and 65%, respectively.

The group of discordant PMBL patients had some special clinical features. They were older than concordant PMBL patients but younger than concordant DLBCL ones. As well as concordant PMBLs, they tended to have local invasion, lung infiltration, fluidothorax and SVCS. On the other hand, more frequent infradiaphragmatic involvement, spleen involvement and less often LDH elevated level made them similar to concordant DLBCLs.

When the analysis of PFS was made, the predictor seemed to better discriminate PMBL patients with superior outcome, although the statistical significance was only marginal (p=0,084). We suppose that our predictor can bring better prognostic information than the clinico-pathological diagnosis. The results should be confirmed on a larger group of patients.

Our results further point out the problem of PMBL diagnosis. As soon as a patient comes with DLBCL histology and mediastinal bulk, clinicians tend to state PMBL diagnosis because more exact guideline is still missing. Gene expression data shows that approximately 25% of these patients are not diagnosed correctly and the course of their disease can be different. Thus, improvement of diagnostic process is needed.

Taken together, using three genes we identified 26% of patients with a mediastinal bulk who did not express a typical PMBL gene profile and 5% patients with expected clinical DLBCL diagnosis who carried a PMBL gene set. These discordant subgroups are numerically fully consistent with the data obtained with microarray techniques [5]. Thus, we suppose it is possible to use microarray data as a source of very valuable data which can be translated into usage of more available techniques, e.g. RTqPCR. An important contribution of this work is the successful use of widely available FFPE material.

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References

[1] SAVAGE KJ Primary mediastinal large B-cell lymphoma. Oncologist 2006; 11: 488–495. doi:10.1634/theoncologist.11-5-488

[2] MARTELLI M, FERRERI AJM, JOHNSON P Primary mediastinal B-cell lymphoma. Crit Rev Oncol Hematol 2008; 68(3): 256–63. doi:10.1016/j.critrevonc.2008.07.020

[3] TRNENY M, VASOVA I, PYTLIK R, BELADA D, JANKOVSKA M et al. The non-Hodgkin's lymphoma subtypes distribution and survival in Czech Republic. Klinicka Onkologie 2007; 5: 340–348. (In Czech).

[4] BOLETI E, JOHNSON PW Primary mediastinal B-cell lymphoma. Hematol Oncol 2007; 25(4): 157–163. doi:10.1002/hon.818

[5] ROSENWALD A, WRIGHT G, LEROY K, YU X, GAULARD P et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med 2003; 198: 851–862. doi:10.1084/jem.20031074

[6] SAVAGE KJ, AL-RAJHI N, VOSS N, PALTIEL C, KLASA R et al. Favorable outcome of primary mediastinal large B-cell lymphoma in a single institution: British Columbia experience. Ann Oncol 2006; 17: 123–130. doi:10.1093/annonc/mdj030

[7] BARTH TF, LEITHÄUSER F, JOOS S, BENTZ M, MÖLLER P Mediastinal (thymic) large B-cell lymphoma: where do we stand? Lancet Oncol 2002; 3: 229–234. doi:10.1016/S1470-2045(02)00714-3

[8] VAN BESIEN K, KELTA M, BAHAGUNA P Primary mediastinal B-cell lymphoma: A review of pathology and management. J Clin Oncol 2001; 19(6): 1855–1864.

[9] SAVAGE KJ, MONTI S, KUTOK JL, CATTORETTI G, NEUBERG D et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. Blood 2003; 102: 3871–3879. doi:10.1182/blood-2003-06-1841

[10] RODIG SJ, SAVAGE KJ, LACASCE AS, WENG AP, HARRIS NL et al. Expression of TRAF1 and nuclear c-Rel distinguishes primary mediastinal large cell lymphoma from other types of diffuse large B-cell lymphoma. Am J Surg Pathol 2007; 31: 106–112. doi:10.1097/01.pas.0000213334.40358.0e

[11] KIMM LR, DELEEUW RJ, SAVAGE KJ, ROSENWALD A, CAMPO E et al. Frequent occurrence of deletions in primary mediastinal B-cell lymphoma. Genes Chromosomes Cancer 2007; 46: 1090–1097. doi:10.1002/gcc.20495

[12] WESSENDORF S, BARTH TF, VIARDOT A, MUELLER A, KESTLER HA et al. Further delineation of chromosomal consensus regions in primary mediastinal B-cell lymphomas: an analysis of 37 tumor samples using high-resolution genomic profiling (array-CGH). Leukemia 2007; 21(12): 2463–2469. doi:10.1038/sj.leu.2404919

[13] FEUERHAKE F, KUTOK JL, MONTI S, CHEN W, LACASCE A et al. NFκB activity, function, and target-gene signatures in primary mediastinal large B-cell lymphoma and diffuse large B-cell lymphoma subtypes. Blood 2005; 106: 1392–1399. doi:10.1182/blood-2004-12-4901

[14] WENIGER MA, GESK S, EHRLICH S, MARTIN-SUBERO JI, DYER MJ et al. Gains of REL in primary mediastinal B-cell lymphoma coincide with nuclear accumulation of REL protein. Genes Chromosomes Cancer 2007; 46(4): 406–415. doi:10.1002/gcc.20420

[15] CHROMCZINSKY P, SACCHI N Single-step method of RNA extraction by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 158–159.

[16] HANS CP, WEISENBURGER DD, GREINER TC, GASCOYNE RD, DELABIE J et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275–82. doi:10.1182/blood-2003-05-1545

[17] ISAACSON PG, NORTON AJ, ADDIS BJ. The human thymus contains a novel population of B lymphocytes. Lancet 1987; 2: 1488–91.

[18] DUNPHY Ch, O'MALLEY DP, CHENG L, FODRIE TY, PERKINS SL et al. Primary mediastinal B-cell lymphoma: detection of BCL2 gene rearrangements by PCR analysis and FISH. J Hematop. 2008;1: 77–84. doi:10.1007/s12308-008-0007-7