

## DIAGNOSTIC DIFFICULTIES IN RECOGNIZING B-CELL LYMPHOMAS IN MEDIASTINAL TUMORS – THREE CASE STUDIES

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### ABSTRACT

**Introduction.** The most frequently diagnosed mediastinal lymphomas include: Nodular Sclerosis Classical Hodgkin Lymphoma (NSCHL) and Primary Mediastinal Large B-cell Lymphoma (PMBL). In the new, 4<sup>th</sup> edition of the *WHO Classification of Tumors of Hematopoietic and Lymphoid Tissue of 2008*, a new category was created: “B-cell Lymphoma, Unclassifiable, with Features Intermediate Between Diffuse Large B-cell Lymphoma and Classical Hodgkin Lymphoma”. It has also been referred to as “Mediastinal Grey Zone Lymphoma”.

**Aim.** The aim of this paper was to analyze morphological and phenotypic characteristics of three diagnostically difficult cases of mediastinal and lymph nodes lymphomas.

**Materials and methods.** Immunohistochemical analysis was performed in two stages: 1) LCA, CD20, CD3, CD30, CD15, Ki67; 2) the panel was extended to include: antibodies Bcl2, Bcl6, CD10, MUM1, CD23, Fascin, transcription factors – PAX5, Oct2, BOB1; LCA/CD45, CD20, CD30, CD15, CD3, CD23.

**Case studies.** The examination encompassed: 2 cases that demonstrated a discordance between the morphology and the phenotype, and 1 case in which two apparently independent neoplastic growths – PMBL and NSCHL – were diagnosed within 4 months. Patients: 2 women (22 and 31 years old) and 1 man (27 years old) – presented large mediastinal masses of diameter larger than 10 cm.

**Discussion.** Differential diagnosis between NSCHL and PMBL is sometimes very difficult. However, NSCHL and PMBL demand different therapeutic strategies. In

the case of PMBL treatment is more intensive. Thus, unambiguous diagnosis is necessary: either NSCHL or PMBL. In some cases, diagnostic difficulties may occur, sometimes it is even impossible to establish diagnosis.

**Conclusions.** Among B-cell lymphomas in mediastinal tumors there are cases of untypical clinical course and untypical morphological and phenotypic characteristics. Thus, it is necessary to re-examine recurrences, including localizations other than the primary one. An adequate, i.e. large enough, specimen taken during mediastinoscopy is the basis for the correct diagnosis. In diagnostically complicated cases, it is necessary to extend the immunohistochemistry panel to include: CD23 and transcription factors: PAX5, Oct2 and BOB1.

**Key words:** nodular sclerosis classical Hodgkin lymphoma (NSCHL), primary mediastinal large B-cell lymphoma (PMBL), mediastinal grey zone lymphoma (MGZL), histopathological diagnosis.

## INTRODUCTION

One of the most frequently diagnosed mediastinal lymphomas include nodular sclerosis classical Hodgkin lymphoma (NSCHL) and primary mediastinal large B-cell lymphoma (PMBL). NSCHL occurs primarily in young adults, with the peak of incidence between 15 and 34 years of age, slightly more frequently in women. It develops in neck and supraclavicular lymph nodes and in approximately 80% of the cases in the anterior mediastinum. Morphological characteristics include “collagenous” fibrosis, the presence of Hodgkin’s cells and lacunar Reed–Stenberg cells (H/RS cells) localized among numerous inflammatory cells.

In the syncytial variant, neoplastic cells form infused areas, especially at the necrosis margins, and mimic neoplasm metastasis or large cell lymphoma metastasis. H/RS cells have a characteristic phenotype and in the majority of cases are: CD30+, CD15+, LCA–, CD3– and CD20– [3, 5, 11, 15]. Prognosis, at the time of modern chemotherapy and radiotherapy, is relatively positive and approximately 75% of patients may be cured [11].

PMBL is a subtype of diffuse large B-cells lymphoma (DLBCL) and accounts for 6–10% of all diagnosed cases [6]. It occurs mostly in young adults, median age – 35 years, twice more often in women. The disease develops as a tumor in the anterosuperior mediastinum. Neck and supraclavicular lymph nodes may be involved. Morphological features of PMBL are diversified. The common features include: diffused proliferation of B-cells of various sizes, from medium to large, fibrosis and compartmentalization [13, 14]. PMBL cells display a positive, strong reaction to CD20+, in approximately 75% of cases the cells show the expression of CD23+, and in about 80% of cases a low expression of CD30+, and are CD15– [6, 12, 16, 18]. Retrospective data covering large numbers of patients indicate a similar or better prognosis in PMBL in comparison to

DLBCL. Recently, common features linking NSCHL with PMBL have been noted, i.e. localization of the tumor mass in anterior mediastinum, frequent involvement of supraclavicular lymph nodes, predominance in young adults, mostly women, fibrotic stroma of the tumor, and many common molecular and genetic features [2, 4, 9, 17, 18, 20]. Sharing common biological and clinical features by NSCHL and PMBL is not surprising, H/RS cells in about 90% of cases are abnormal B-lymphocytes [10, 15]. NSCHL and PMBL are, however, treated differently. The application of a proper therapy from the beginning of the treatment greatly affects survival. Consequently, unambiguous diagnosis is necessary: either NSCHL or PMBL. Sometimes there occur diagnostic difficulties and differential diagnosis may be impossible. For such clinical pictures a new category was created in the new, 4<sup>th</sup> edition of the WHO Classification of 2008: “B-cell Lymphoma, Unclassifiable, with Features Intermediate Between Diffuse Large B-cell Lymphoma and Classical Hodgkin Lymphoma”. Generally it refers to a large mediastinal tumor, thus, this entity is also known in literature as “mediastinal grey zone lymphoma” (MGZL). It occurs in young adults, more frequently in men 20–40 years old. A large tumor mass in the mediastinum leads to symptoms of superior vena cava syndrome and respiratory failure. Morphological and phenotypic characteristics are varied, and as follows from the definition of this entity, morphological and phenotypic features of classical Hodgkin lymphoma (CHL) and PMBL overlap [8, 17, 18, 20]. Clinical course is not as yet certain, but it seems to be more aggressive than CHL and PMBL, and with a worse prognosis. There are no standard treatment methods, but in some cases chemotherapy like the one used in aggressive B-cell lymphomas is recommended [9].

## AIM

The aim of this paper is to analyze morphological and phenotypic characteristics of three diagnostically difficult cases of mediastinal and lymph nodes lymphomas.

## MATERIALS AND METHODS

The examination encompassed: 2 cases that demonstrated a discordance between the morphology and the phenotype, and 1 case in which two apparently independent neoplastic growths – PMBL and NSCHL – were diagnosed within 4 months. Patients: 2 women (22 and 31 years old), 1 man (27 years old) – presented large mediastinal masses of diameter larger than 10 cm. The women also revealed enlarged lymph nodes. The specimens taken for examination were obtained from patients treated in the Ministry of Internal Affairs and Administration Hospital in Olsztyn and diagnosed in the Pathomorphology Unit of the Provincial Specialist Hospital in Olsztyn.

The specimens were taken from the mediastinal tumors and lymph nodes, fixed in 10% buffered formalin solution, routinely treated and sunk in paraffin. Paraffin blocks were sliced into 4  $\mu$ m sections, which were transferred to microscope slides and stained with hematoxylin and eosine.

Immunohistochemical analysis was performed in two stages. In the 1<sup>st</sup> stage the following tests were carried out: LCA, CD20, CD3, CD30, CD15, Ki67.

In the 2<sup>nd</sup> stage the panel was extended to include:

- antibodies Bcl2, Bcl6, CD10, MUM1, CD23, Fascin, transcription factors – PAX5, Oct2, BOB1;
- LCA/CD45 – common lymphocyte marker;
- CD20, Bcl2, Bcl6, CD10, MUM1 – common B-cells markers and/ or their functional stages;
- PAX5, BOB1, Oct2 – transcription factors of B-cells differentiation;
- CD30 – lymphocyte activation marker, H/RS cells marker in HL;
- CD15 – H/RS cells marker in CHL and myeloid cells marker, occurring in some DLBCL;
- CD3 – T-lymphocyte marker;
- CD23 – thymic B-cells marker;
- Fascin – dendritic cell and RS cell marker.

## CASE STUDIES

### Case 1

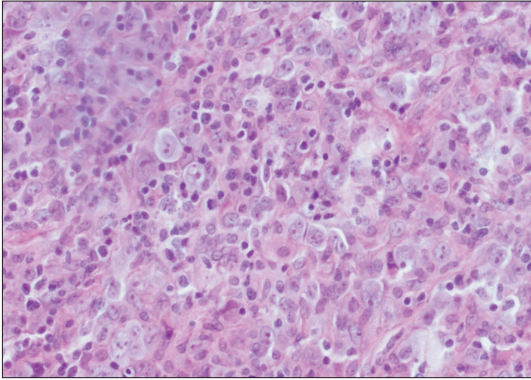
The patient, M.D., 22 years old, was admitted to the Ministry of Internal Affairs and Administration Hospital in Olsztyn in April 2006, diagnosed in December 2005 with Hodgkin lymphoma NSII CS IIXB. The patient had previously received three courses of chemotherapy according to the ABVD scheme, leading to disease stabilization (DS). On admission it was revealed: left supraclavicular lymph nodes in fixed packages (35 mm), and in computed tomography (CT) of the chest a nodular mass in the mediastinum (88 × 63 × 126 mm). In laboratory tests: ESR 110mm/h, LDH – not assayed. In the 1<sup>st</sup> stage of treatment, the patient received chemotherapy, 3 × ABVD/ADM, leading to disease stabilization (DS). Supraclavicular lymph node was taken to verify diagnosis. Morphological characteristics of the node were in line with the CHL, nodular sclerosis NSII, syncytial variant. Layers of collagen divided the node tissue into nodules. Some nodules showed infused areas of atypical, large cells with acidophile nucleoli. Some of these cells might have been morphologically in line with H/RS cells. Inflammatory cells were numerous. Diffused necrosis areas with large, atypical cells forming infused areas around them were noticeable. Immunohistochemical characteristics were different from what we had expected. Tumor cells showed CD20+ expression. The intensity of reactivity varied in various areas of the node. Around necrosis foci, the majority of large cells were CD20+, but their reactivity was weaker than that of B-lymphocytes. There were also nodules where all tumor cells showed uniform, high CD20+ reactivity. The expression of CD15+ was also noticeable; it was strong, on the membrane and perinuclear, alike in all cells; whereas the expression of CD30+ (on the membrane and perinuclear) was very weak in selected cells. On the basis of the morphological and

phenotypic characteristics (CD15+, CD30+) in the lymph node we diagnosed Hodgkin lymphoma, nodular sclerosis with atypical phenotype CD20+. In the 2<sup>nd</sup> stage, patient was qualified for HDT + ASCT. She received chemotherapy according to the following schemes:

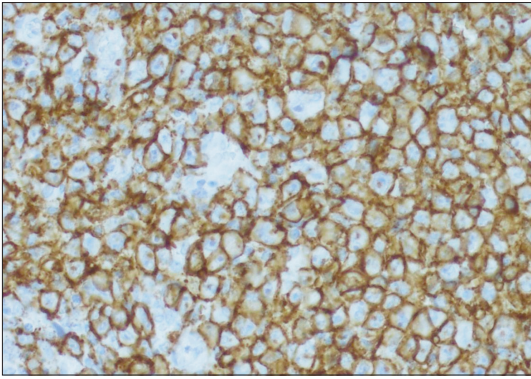
- a) May 8–22, 2006: 2 × ICE. CT revealed progression of the mediastinal mass to 110 × 65 × 140 mm; liquid in the left pleural cavity; left supraclavicular node 23 × 24 mm with disintegration;
- b) June 12 – August 16, 2006: 4 × EPOCH. CT showed progression in the form of mediastinal tumor enlargement, nodular progression in the right and left supraclavicular areas, metastasis foci in lungs;
- c) 1 × CN3OP. Clinically, general symptoms intensified (fever, night sweats, itching) and did not cease despite steroid therapy.

The patient was disqualified from non-standard treatment with CD34+ cells transplantation from non-related donor. Due to a rapid, symptomatic progression of lesions in the mediastinum, the patient received palliative radiotherapy focused on the tumor area. After 3 months, further progression occurred. The patient died in February 2007, 3 months after the completion of radiotherapy and 15 months after diagnosis. When it was possible to perform more detailed immunohistochemical tests in November 2009, the antibodies panel was extended to include transcription factors and Bcl2, Bcl6, MUM1, CD23, Fascin and Ki67. Neoplastic CD20+ cells showed also positive reactions to PAX5 and Oct2, with the intensity comparable to that of B-lymphocytes reactivity. The cells did not display staining when reacting with BOB1. In reacting with Fascin, all neoplastic cells showed a strong, cytoplasmic positive reaction. A positive, strong reaction to MUM1 and Bcl2 was revealed in the majority of the cells. And, what should be emphasized a strong, positive reaction to CD23 was displayed. The last reaction is more typical of PMBL, where it occurs in about 75% of the cases [1]. It has been also described to occur in CHL, but far more rarely. Mitotic activity – Ki67 occurred in about 50% of the neoplastic cells. The cells showed negative reactions to CD10, Bcl6, CD3 markers.

In the described case, a discordance between the morphological characteristics indicating NSCHL and phenotypic characteristics with a strong expression of CD20 in line with large B-cells lymphoma can be noticed. Finally, the case was qualified as MGZL (Fig. 1, 2).



**Fig. 1.** Case 1. Lacunar cells and inflammatory background [HE 200×]



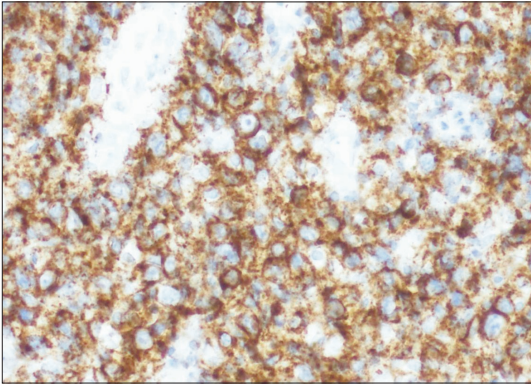
**Fig. 2.** Case 1. CD20 is strongly positive in the neoplastic cells [Magn. 200×]

## Case 2

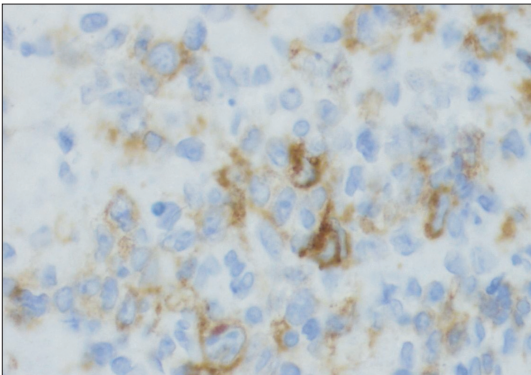
The patient, W.S., 27 years old, was diagnosed in October 2007 due to dry cough, effort dyspnea with lymphadenopathy in the supraclavicular area. Clinically: superior vena cava syndrome and both supraclavicular lymph nodes enlarged to 20 mm were revealed. CT of the anterior mediastinum revealed: large heterogeneous pathological mass 136 × 85 mm, extending in its anterior part the mediastinum and penetrating the wall of the chest involving interior pectoral muscle. General symptoms were present – night sweats. Laboratory tests revealed: ESR – 109/115 mm/h, LDH – not assayed. Uric acid and blood picture within normal range. Histopathological examination was performed on small specimens of the mediastinal tumor. Morphological characteristics were in line with CHL, nodular sclerosis NSII or LD. Pleomorphic, “sacomatous” H/RS cells were present, forming foci on fibrotic stroma. They were accompanied by small lymphocytes and a small number of neutrophil granulocytes. In some specimens there were thick collagen strands. Immunohistochemical characteristics were surprising – large cells showed strong positive reactions to CD20+, weak to CD30+ and weak to CD15+ in rare cells. On the basis of the morphological and phenotypic characteris-

tics (CD30+, CD15+), the mediastinal tumor was recognized as Hodgkin lymphoma, nodular sclerosis G2 with an expression of CD20+. From November 11, 2007 to April 4, 2008, the patient received 4 × ABVD, and due to the disease progression, from July 7 to July 31, 2008, chemotherapy according to the 2 × ICE scheme. From the onset of the treatment, the disease was resistant to the administered chemotherapy. The patient was qualified for non-standard procedure of CD34 cells transplantation from a related donor. The patient died during the preparation for the transplantation procedure due to disease progression 15 months from its diagnosis in February 2009.

In additional IHC tests, neoplastic cells showed a strong expression of PAX5 and Oct2 (like reactive B-lymphocytes) and in some cells a weak expression of BOB1. The majority of neoplastic cells were Bcl2+, Bcl6+, Fascin+. All cells were MUM1+. Mitotic activity was high – Ki67 in about 75% of the cells. Negative reactions were revealed to anti-CD10 and CD23 antibodies. Additionally, we performed a test with Epstein-Barr virus antibody (Epstein-Barr virus – latent membrane protein, EBV LMP), and the reaction was negative. Additional immunohistochemical tests, with Oct2+, BOB1+, Bcl6+, may suggest PMBL or MGZL (Fig. 3, 4).



**Fig. 3.** Case 2. CD20 is strongly positive in the neoplastic cells [Magn. 200×]



**Fig. 4.** Case 2. CD30 is strongly positive in some neoplastic cells [Magn. 200×]

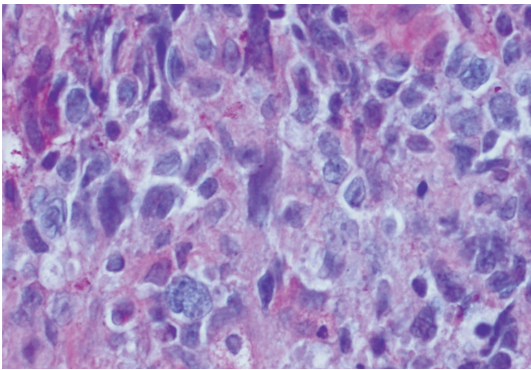
### Case 3

A 31-year-old patient, J.P., was diagnosed in January 2005 due to effort dyspnea, cough, periodic hemoptysis. The patient manifested general symptoms such as: night sweats and fever. Physical examination revealed: both supraclavicular lymph nodes enlarged to 15 mm, left axillary lymph node to 30 mm, and CT revealed: mediastinal tumor of 104×11×130 mm. Laboratory tests: raised ESR level to 80 mm/h, LDH 1280 (N; to 450). Bronchoscopy revealed: tumor occluding the aperture of the right upper and intermediate bronchus from which specimens were taken for histopathology testing. The morphological and phenotypic characteristics were in line with PMBL. In fibrotic stroma there were large cells showing a strong expression of CD20+, CD30+ (in a small number of cells), CD15- and a strong mitotic activity Ki67++++. The specimens also presented necrotic tissue and numerous *Aspergillus fumigatus* colonies.

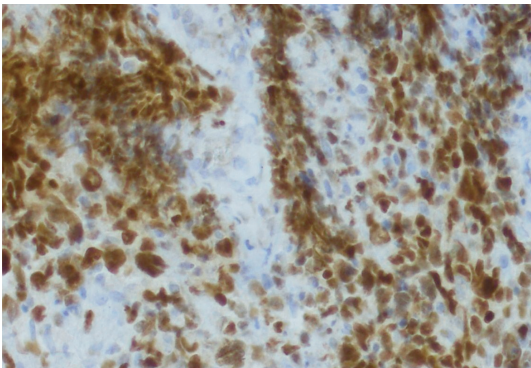
In the mediastinal specimen primary mediastinal large B-cell lymphoma was diagnosed. From February 9, 2005 to April 14, 2005, the patient received chemoimmunotherapy according to the 4×R-CHOP scheme, 21-days rhythm. Simultaneously, she received antifungals (Fluconazole). In control CT after 4 courses of chemotherapy, disease stabilization was manifested. After 4 months, during chemotherapy of the 1<sup>st</sup> stage a specimen was taken for histopathology analysis: left axillary lymph node. The morphological characteristics were in line with the syncytial variant of NSCHL. Neoplastic cells showed a strong expression of CD30+ (membrane and perinuclear activity), expression of CD20+, alike in all cells, but weaker than in the surrounding reactive B-lymphocytes. Large cells were LCA- and CD15-. In the node it was recognized: CHL, nodular sclerosis, G2. Because of no reaction to chemotherapy of the 1<sup>st</sup> stage, the patient was qualified for ASCT+HDT – high dosage chemotherapy supported with transplantation of autologous hematopoietic cells. From May 6, 2006 to August 8, 2006, the patient received chemotherapy of the 2<sup>nd</sup> stage according to the 4×ICE scheme, 21-days rhythm. Imaging examinations showed only disease stabilization. Following apheresis of CD34+ cells while waiting for HDT+ASCT procedure, the patient received maintenance treatment according to the 5×CN3OP scheme, 21-days rhythm. Due to the activation of *Aspergillus fumigatus* infection, the patient received antifungal medication (voriconazole) until fungal infection was eradicated. At that time the neoplasm progressed. The patient received 4×ESHAP, 21-days rhythm. From October 13 to 17, 2006, the patient received chemotherapy according to the BEAM scheme, supported by the transplantation of autologous hematopoietic cells (October 20, 2006). PET showed complete remission. In January 2009, progression of the disease was found in the chest (CT and PET examinations). The patient refused consent to confirm the diagnosis via thoracotomy. From February 2009 the patient received 6×ABVD, achieving complete remission confirmed by PET. From October 19 to November 14, 2009, the patient received radiotherapy focused on the changes in the chest. Follow up PET (March 2010) is ambiguous – post-radiation changes or active pleonasm. The patient remains under observation – control ex-



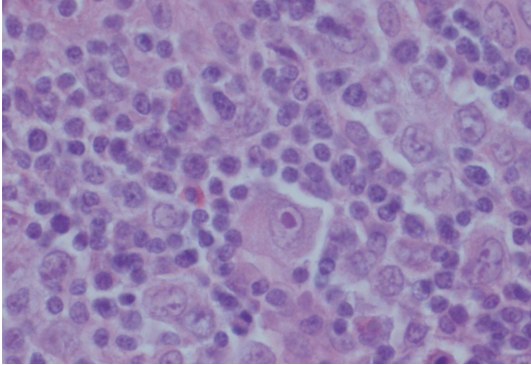
amination is scheduled after 3 months. Additional ICH analysis performed in September 2009 confirmed the diagnosis of mediastinal PMBL, i.e. large cells were: LCA(CD45)+, CD20+, PAX5+, Oct2+, BOB1+, Bcl2+, MUM1+. A small number of cells showed a positive membrane expression of CD30+. Mitotic activity was high – Ki67+ in about 85% of the cells. Pleonasm cells reacted negatively to the following markers: CD15, Fascin, Bcl6, CD10 and CD23. Additional, supplementary examinations of the node also confirmed the previously established diagnosis of Hodgkin lymphoma. Transcription factors Oct2 and BOB1 were negative, PAX5 was positive, but considerably weaker in comparison to reactive B-cells. In the majority of the cells the expression of Fascin, MUM1, Bcl2 was observed and of CD23 in about 30% of the cells. Mitotic activity was high – Ki67+ in about 70% of the cells. There were negative reactions with: Bcl6, and CD10 markers. Within 4 months the patient was diagnosed with PMBL in the specimen of the tumor occluding the bronchus and NSCHL in the axillary lymph node. Thus, there were either two simultaneously progressing neoplastic processes or the case could be classified as MGZL, according to the definition provided by the new WHO classification, so NSCHL in the node should be seen as PMBL recurrence (Fig. 5–8).



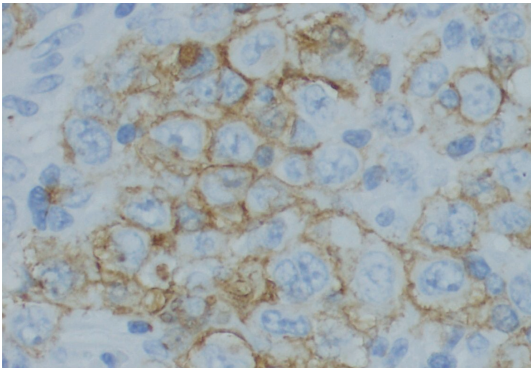
**Fig. 5.** Case 3. PMBL. Morphologically PMBL displays significant pleomorphism [PAS 400×]



**Fig. 6.** Case 3. PMBL. PAX5 is strongly positive in the neoplastic cells [Magn. 200×]



**Fig. 7.** Case 3. NSCHL. Lacunar cell and inflammatory background [HE 400×]



**Fig. 8.** Case 3. NSCHL. CD20 is positive in the neoplastic cells [Magn. 400×]

## DISCUSSION

Differential diagnosis between NSCHL and PMBL is sometimes extremely difficult. In routine immunodiagnosics of CHL the following panel of antibodies is recommended: CD45, CD30, CD15, CD20, CD3 [3, 5, 11, 15]. It is, however, insufficient in diagnostically difficult cases such as the syncytial variant of NSCHL, especially when the specimen examined is small. Such a specimen may consist only of necrosis with the neoplastic cells surrounding it, in which it may be extremely difficult, if not impossible, to recognize diagnostic H/RS cells. If neoplastic cells are CD30+ and CD15+, but LCA- and CD20-, NSCHL diagnosis is obvious. However, H/RS cells show the expression of CD20+ in about 30% of all CHL cases [5, 11, 15] but the intensity of the reaction is generally weaker in comparison to reactive B-lymphocytes and it is heterogeneous. Such a picture of CD20 reaction in neoplastic cells, with a simultaneous positive membrane and perinuclear activity of CD30 and CD15 strongly indicates NSCHL. It happens, however, that the cells are CD20+ and CD30+, but CD15-, or CD20+ and

CD15+, but CD30-. Then, it is helpful to extend the IHC panel to include the following antibodies: transcription factors PAX5, Oct2, BOB1 and fascin [3–5, 10, 11, 15, 18]. PAX5 is positive in the majority of Hodgkin lymphoma cases, while BOB1, Oct2 are usually negative or, rarely, only one of them shows a weak expression. Fascin may also be helpful as it is a good marker of H/RS cells, giving a strong cytoplasmic reaction. Only 15% of non-Hodgkin lymphomas show positive staining for Fascin.

The morphological characteristics of PMBL are very diverse [6, 12–14, 16]. Specimens taken from mediastinal tumors via mediastinoscopy or surgical biopsy are often small and may be crushed. In the biopsy material all histological components of PMBCL are not always visible. Thus, if neoplastic cells look similar to H/RS cells, and fibromatosis is in the form of thick collagen strands instead of the expected thin connective tissue fibers, differential diagnosis with NSCHL is very difficult. PMBL cells have positive B-cells reactions such as CD20 and CD79a, and they are strong and identical in all cells, with a simultaneous strong expression of CD45(LCA). CD30 is present in about 80% of the cases, expression is however weak and uneven in comparison to that in Hodgkin lymphoma. A positive reaction to CD15 is present sporadically [6, 12, 16]. Tumor cells frequently display a positive reaction to MUM1 and CD23 in about 75% [1]. Reaction to Bcl2 is positive in 55–80%, and to Bcl6 in 45–100% [5, 8]; CD10 is rarely positive – in 8–32% [5, 8]. Transcription factors: PAX5, Oct2, BOB1 are positive [6, 12, 16]. It should be underlined that secondary involvement of the mediastinum in the course of DLBCL is more frequent than its primary involvement in PMBL. Consequently, differential PMBL diagnosis must encompass both NSCHL and DLBCL. Clinical data is also very important. Young age of the patient, involvement of the mediastinum, and potentially of supraclavicular nodes, without the involvement of other nodes and marrow, with a typical morphological characteristics of fibrosis indicate PMBL.

Cases described in literature as “grey zone lymphomas” or “large B-cell lymphomas with Hodgkin’s features” [7] represent tumors which have both PMBL and CHL features. The new category in the latest WHO classification “B-cell Lymphoma, Unclassifiable, with Features Intermediate Between DLBCL (Diffuse Large B-cell Lymphoma) and Classical Hodgkin Lymphoma” refers to such cases. They occur in the mediastinum but may also occasionally appear in other localizations. With regards to mediastinal tumors, recognizing grey zone lymphoma should be strictly limited to cases with overlapping features of PMBL and CHL, especially when there is a discordance between the morphological and immunophenotypic characteristics [2, 7–9, 17–19]. Two new subgroups have been identified in this respect:

1. Cases morphologically similar to CHL but with atypical features such as a large number of large mononuclear cells, no typical inflammatory setting and immunohistochemical reactions atypical for CHL – strong expression of CD20 and expression of CD79a;

2. Cases morphologically in line with PMBL but with phenotypic characteristics and features of CHL. The cells show weak reactions to CD20 or no reactions at all, but CD15 expression, if only focal, is noted in about 60% of the cases. Transcription factors of B-cells (PAX5, Oct2, BOB1) are usually positive, although often weak.

Complex lymphomas – NSCHL and PMBL and consecutive lymphomas, generally in the order of CHL first followed by PMBL are also classified as grey zone lymphomas, although it is not certain whether their biology is connected with lymphomas of overlapping features of CHL and PMBL. The creation of a new category – grey zone lymphoma, has taken the burden off pathologists in terms of responsibility in recognizing cases earlier impossible to diagnose unambiguously. The inclusion of a particular case to that group should be, however, careful and only after excluding all means to classify it as NSCHL or PMBL [8]. The extension of the IHC panel is absolutely vital.

## CONCLUSIONS

1. Malignant B-cells lymphomas in mediastinal tumors involve cases of atypical clinical courses and atypical morphological and phenotypic characteristics.
2. It is necessary to re-examine recurrences, including other localizations than the primary ones.
3. Adequate, i.e. large enough, specimen taken during mediastinoscopy is the basis for the correct diagnosis.
4. In diagnostically difficult cases it is necessary to extend the immunohistological panel to include CD23 and transcription factors: PAX5, Oct2 and BOB1.

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