Case Report
The t(14;18)(q32;q21) with extra MYC signal - is it a gray zone lymphoma?

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Abstract: Double-hit lymphomas (DHL) are defined as B-cell lymphoma with a chromosomal breakpoint affecting the MYC/8q24 locus in combination with rearrangement at (14;18)(q32;q21). We recently observed three cases of B-cell lymphoma with an extra intact MYC signal in association with the t(14;18)(q32;q21) translocation. The impact of an extra copy of MYC to the clinical course and prognosis of one patient with Diffuse Large B Cell Lymphoma (DLBCL) and two patients with Follicular Lymphoma (FL) was evaluated. Flow cytometry in all cases demonstrated lambda- or kappa-light chain restricted CD20 and CD10 positive neoplastic B cells. FISH analysis was negative for MYC gene rearrangement but demonstrated an extra copy of intact MYC. Tissue sections displayed typical starry sky "gray zone" lymphoma morphology in case of DLBCL and FL morphology in cases 2 and 3, with high Ki67 labeling in all three cases. All patients responded well to initial chemotherapy although displayed variant outcome after initial remission. The patient with DLBCL deceased within a year of diagnosis while the other two patients with FL showed much better overall survival. Our limited experience showed that additional copy of intact MYC may be equivalent to "classic" DHL on the background of DLBCL with additional cytogenetic abnormalities, however isolated t(14;18)(q32;q21) translocation in combination with additional copy of intact MYC may demonstrate histology and clinical outcome more comparable with "classic" low grade follicular lymphoma, albeit with more aggressive morphology.

Keywords: Double hit lymphoma, MYC, translocation, amplification, follicular lymphoma

Introduction

So called "Double-hit lymphoma" (DHL) or Gray zone lymphomas are referred to mature B-cell lymphomas often bearing concurrent chromosomal rearrangement of MYC/8q24 locus in association with t(14;18)(q32;q21) translocation involving BCL2 gene at 18q21 [1, 2]. The t(14;18)(q32;q21) translocation can be detected in low grade FL as well as in some high grade FL and DLBCL. The latter is usually associated with other genetic abnormalities [3]. Most DHL cases are diagnosed as "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BL)" based on 2008 WHO classification. In general, DHL display aggressive clinical courses, do not respond well to chemotherapy, and are associated with poor outcome. Activation of BCL2 promotes resistance to apoptosis, while activation of MYC provokes continuous, mitogen-independent proliferation of cells, resulting in highly aggressive tumor resistant to therapy [1, 2]. Of notice, activation of MYC may occur through diverse mechanisms including translocations, amplifications or altered stability [4]. While combination of t(14;18)(q32;q21) with translocation of MYC to the gene of immunoglobulin light chain (IGH) resulting in highly aggressive DHL is relatively well studied, extra copy of c-MYC only recently attract attention as possible mechanism of "second hit" resulting in gray zone lymphomas [5-7]. It is well known that extra copy of HER2NEU is associated with more aggressive breast cancer and is targeted for therapeutic intervention. Limited data are available on the role of extra copy of c-MYC in gray zone lymphomas. It is not clear whether extra copy of c-MYC is equivalent to c-MYC translocation. Some literatures suggested grave prognosis for the lymphomas with extra MYC signal plus IGH/BCL2 translocation [5, 7] while other studies did not demonstrate correlation between the copy number of MYC

and disease outcome [6]. We believe that further studies are necessary to explore the prognostic implications of lymphomas with IGH/BCL2 translocation and extra copy of cMYC. Here we reported three B-cell lymphoma cases with t(14;18)(q32;q21) and one extra copy of intact c-MYC on the background of DLBCL and FL. Histology, immunohistochemistry and clinical course of the disease were discussed.

Materials and methods

Case selection

Three cases of B-cell lymphoma with t(14;18) and an additional copy of MYC were collected from Department of Pathology, Truman Medical Center, Kansas City, MO. Corresponding medical records were reviewed to obtain clinical information including sites of involvement, treatment regimens, response to therapy, and overall survival.

Histology and immunohistochemistry

The morphology was studied on formalin-fixed and paraffin-embedded tissue section, peripheral blood smear, cerebrospinal fluid (CSF), endoscopic ultrasound (EUS) guided fine needle aspiration (FNA), and bone marrow biopsy. The CSF was collected via lumber puncture. Wright-stained cytospin preparation was made for the CSF specimen. The Wright-stained preparation and Papanicolaou-stained preparation was made for the FNA specimen. The Wright-stained preparation was made for bone marrow aspiration. Immunohistochemical staining was performed via an automated immunostainer Ventana according to established protocols. Positive and negative controls were run with each case.

Flow cytometric immunophenotyping

The specimensprocessing and antibody staining was performed according to manufacture recommendation by Clarient, Inc. The antibody panel included CD10, CD11c, CD14, CD16, CD19, CD2, CD20, CD22, CD23, CD3, CD38, CD4, CD45, CD5, CD56, CD7, CD8, FMC7, Kappa light chain, Lambda light chain. The cell population analyses were performed on 8 colors Gallios Beckman Coultier flow cytometer at Clarient Inc. and interpreted at Truman Medical Center.

### Chromosome analysis and fluorescence in situ hybridization (FISH)

Genetic analyses were performed at Children's Mercy Hospital Clinical Cytogenetics Laboratory. Chromosome analyses were performed with cell culture stimulated for mitosis. The cells are then harvested and stained to produce G-bands. Metaphase chromosomes are viewed microscopically and aligned in a standard sequence based on size, centromere location, and banding pattern.

FISH probe Cytocell IGH (14q32.3) and BCL2 (18q21.3) dual fusion probes was used to show IGH/BCL2 gene fusion. Poseidon MYC (8q24.1) break-apart probes and Cytocell BCL6 (3q27) break-apart probes were used to test rearrangement, gain or loss of copy number of MYC gene and BCL6 gene. The specimen processing and hybridization were performed according to manufacturers' recommendation. The samples were analyzed using standard fluorescence microscopy methods. The cytogenetic analyses were performed at Children's Mercy Hospital and Clarient Inc.

Results

The patient of the first case was a 60-year old Caucasian male presenting with pain in left shoulder and abdomen associated with night sweat for two months and weight loss of 20 pounds in one month. Multiple enlarged lymph nodes were found in left axilla by ultrasound. Computed tomography scans revealed extensive lymphadenophy in the chest, abdomen, and pelvis. X-ray displayed fracture of proximal humerus on the left side. Involvement of bone marrow and central nervous system were shown via the intermedullary biopsy from the fracture site and CSF preparation. The diagnosis of B-cell lymphoma, unclassifiable, intermediate between DLBCL and BL was made. The patient completed 7 cycles of chemotherapy including CALGB 10002 protocol for 4 cycles, one cycle of doxorubicin, rituximab, and cyclophosphamide followed by 2 cycles of R-CHOP without vincristine. He developed methotrexate myleopathy after 4-cycle CALGB 10002 protocol. The patient demonstrated remission at the completion of chemotherapy although hepatosplenomegaly persisted. Bone marrow biopsy and CSF were negative for residual lymphoma involvement. However, he relapsed 4 months
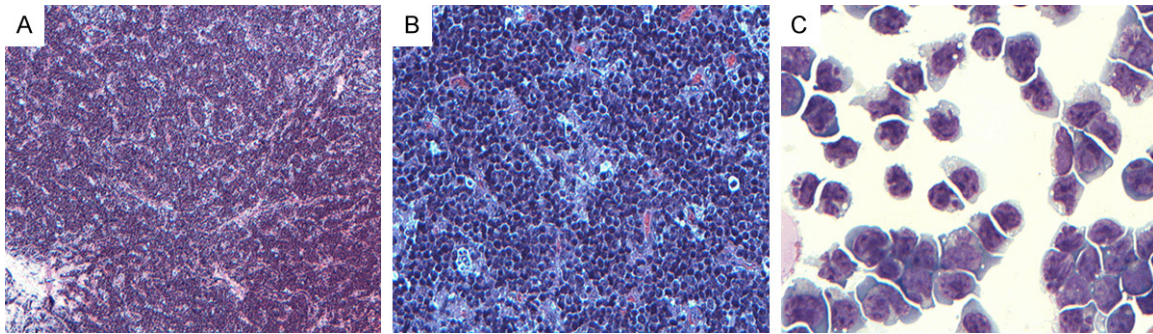
Figure 1. Case 1: H&E stained sections of the lymph node biopsy demonstrated diffuse proliferation of medium- to large-sized, lymphoid cells that completely effaced the architecture of the lymph node. A "starry sky" growth pattern with interspersed macrophages was displayed. 100 × (A) and 400 × (B). Cytologic preparation from cerebral spinal fluid revealed occasional vacuoles in cytoplasm. 1000 × (C).
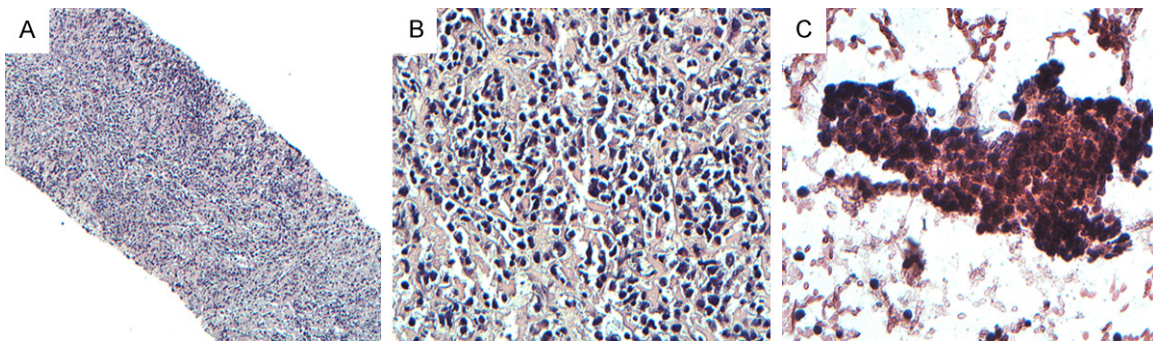
Figure 2. Case 2: H&E stained sections of the lymph node biopsy demonstrated diffuse proliferation of small- to medium-sized lymphoid cells with bands of fibrotic sclerosis. 100 × (A) and 400 × (B). Cytologic preparation of fine needle aspiration displayed clusters of abundant lymphoid cells. 400 × (C).

later with lymphomatous infiltration of the sacral plexus. CSF showed the presence of malignant cells of B-cell lymphoma as well. He was treated with one cycle salvage chemotherapy RICE (Rituximab, Ifosfamide, carboplatin and etopside). He did have persistent pancytopenia and recurrent infection that precluded administration of further chemotherapy. His lymphoma progressed further and he was transitioned to hospice care before he died from his disease. His remission interval for B-cell lymphoma was only 4 months. The patient's condition worsened rapidly since recurrence.

The second patient was a 66-year old, African American male presenting with right upper quadrant abdominal pain for about 5 weeks in association with weight loss of 15-20 pounds, occasional night sweats, early satiety, dark urine, and scleral icterus. His abdominal CT scan had revealed a 7 × 11.5 × 8 cm pancreatic head mass with periportal lymphadenopathy. The patient was diagnosed with FL, Grade

3. He received one cycle of R-CHOP. Then he was switched to dose adjusted EPOCH-R after the C-MYC testing came back positive. He completed 6 cycles of dose adjusted EPOCH-R and went into complete remission. He is now 15 months after completion of his treatment and he continued to be in remission with no evidence of disease relapse.

The third patient is a 48-year old African American male presenting with refractory and worsened back pain and 27-pound weight loss during 6 months. He had a long-standing history of chronic back pain due to a trauma occurring 20 years ago. The MRI of lumbar spine demonstrated a retroperitoneal mass which was partially shown in the image. The following CT of the abdomen and pelvis displayed multiple intra-abdominal lymphadenopathies including a bulky 16 cm retroperitoneal lymphadenopathy. The patient was diagnosed with FL, Grade 1 with bone marrow involvement. He was subjected to chemotherapy of 6 cycle of R-
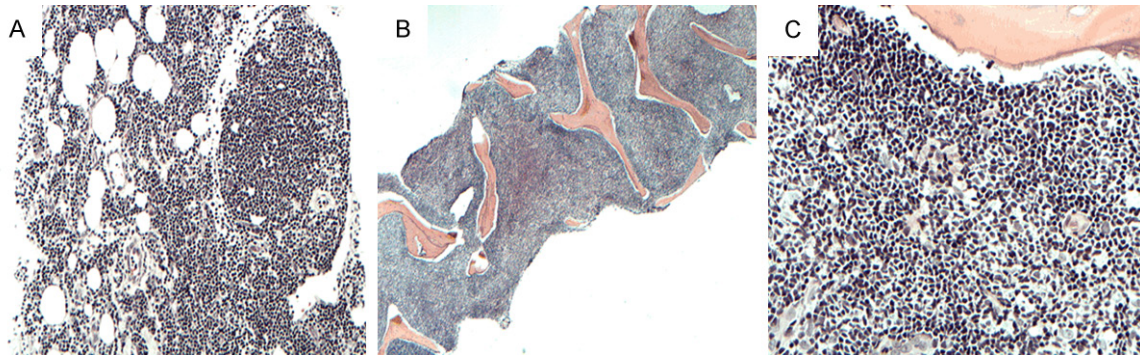
Figure 3. Case 3: H&E stained sections of the lymph node biopsy demonstrated diffuse proliferation of small- to medium-sized lymphoid cells with nodular growth pattern in some areas. 200 × (A). H&E stained section from bone marrow biopsy showed para-trabecular condensation similar to follicular lymphoma. 100 × (B) and 400 × (C).
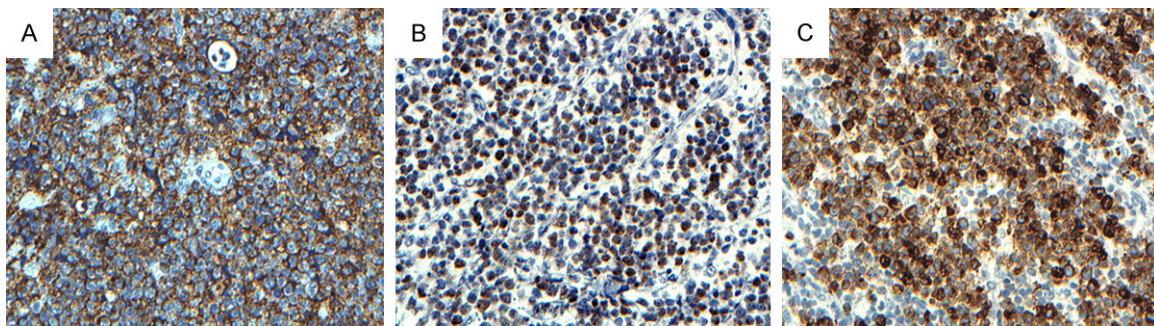
Figure 4. Representative immunohistochemical staining from case 1 demonstrated cytoplasmic positivity of CD20 (A), nuclear positivity of BCL6 (B), and nuclear positivity of BCL2 (C) in the tumor cells.

CHOP and will be maintained with Rutiximab for 2 years. He demonstrated significantly improvement on back pain and tolerated very well with the chemotherapy.

*Morphologic and immunohistochemical findings*

Histologic examinations of the lymph node biopsies in all three cases demonstrated effacement of the lymph node architecture and diffuse proliferation of monotonous lymphoid cells. The neoplastic cells had moderate amount of deeply basophilic cytoplasm and round nuclei, some with multiple nucleoli. The first case displayed proliferation of enlarged lymphoid cells interspersed by macrophages in a "starry sky" pattern. Concurrent CSF smears demonstrated lymphocytes with multiple cytoplasmic lipid vacuoles, similar to Burkitt lymphoma cells. Numerous mitotic figures were present (**Figure 1**). The second case demonstrated diffuse proliferation of monotonous medium size lymphocytes with irregular hyper-

chromatic nuclei and inconspicuous nucleoli and scant pale cytoplasm within bands of fibrotic sclerosis. Increased number of mitotic figures was noted (**Figure 2**). The third case showed proliferation of small to medium lymphocytes with irregular nuclei and inconspicuous nucleoli scant pale cytoplasm consistent with centrocytes which demonstrated nodular growth pattern in some areas (**Figure 3**). Cytoplasmic lipid vacuoles were not observed in Case 2 or 3. Although scattered foamy macrophages were noted, obvious "starry sky" pattern was not appreciated. Bone marrow biopsies from Cases 1 and 3 showed extensively replaced by proliferation of the lymphoma cells with a condensation of para-trabecular area in case 3.

Immunohistochemical studies demonstrated positivity of tumor cells for CD20, CD10, BCL6 and BCL2 (**Figure 4**). TdT and CD 23 were negative in the tumor cells. CD3 and CD5 highlighted background T lymphocytes. Ki67 stains showed immunoreactivity in more than 95%, 40%,
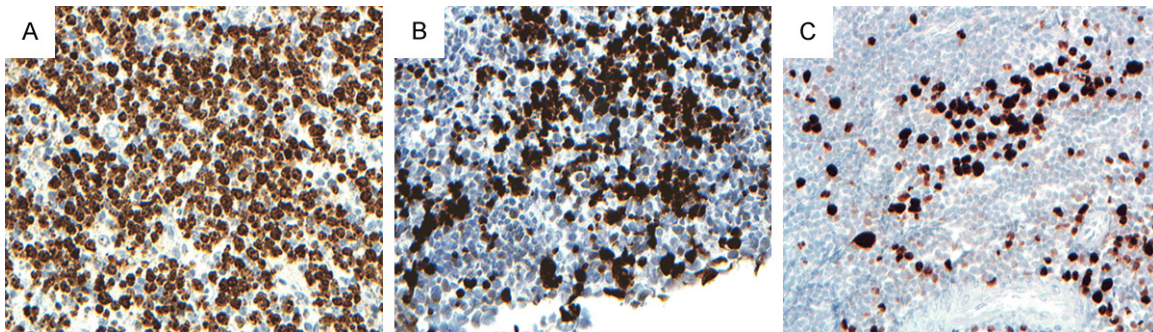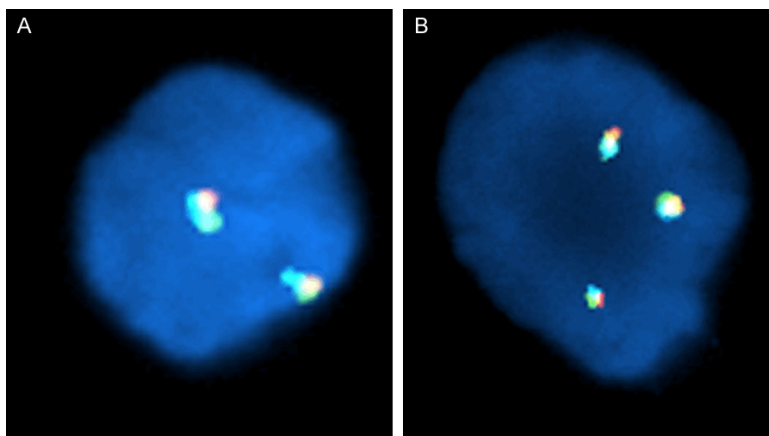
Figure 5. Immunoreactivity of Ki67 in the tumor cells demonstrated proliferation index of 95% in case 1 (A), 40% in case 2 (B), and 25% in case 3 (C).
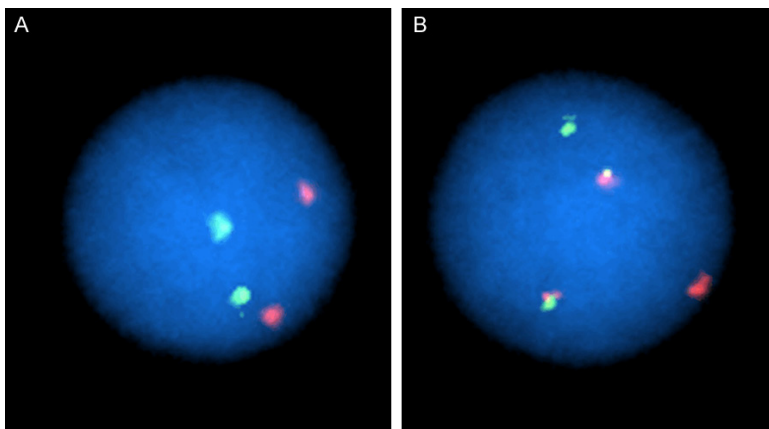
Figure 6. Representative picture of dual fusion probes demonstrated the normal control (A) and the translocation t(14;18) in the tumor cells (B). The probe labeled with red fluorescence targeted IGH (14q32.3) and the probe labeled with green fluorescence targeted BCL2 (18q21.3).

Figure 7. Representative picture of break-apart probes targeting MYC (8q24.1) demonstrated the normal control (A) and 3 intact copies of MYC in the tumor cells (B).

Flow cytometry findings

Flow cytometry analysis was performed on different specimens including lymph node biopsies, peripheral blood, CSF, pancreatic FNA and bone marrow from the three cases. The results have shown a population of B-cells with monotypic surface immnunoglobulin light chain of either kappa or lambda. These neoplastic cells co-expressed CD10, CD19, and CD20, consistent with B-cell non-Hodgkin lymphoma of follicle center cell origin.

FISH and cytogenetics

FISH analysis demonstrated IGH/BCL2 gene rearrangement and three copies of MYC without MYC gene rearrangement in all three cases (**Figures 6**, **7**).

Cytogenetic study demonstrated additional abnormal findings in Case 1, including abnormal male complex karyotype including 47, XY, +X, t(14;18)(q32.3;q21), 48, sl, der(1) t(1;8)(p36.3;q23), +12, del(13)(q13q33), 48, sdl1, t(X;7)(q24;p22), 48, sdl2, del(3q27), 46, XY. The chromosome analysis displayed t(14;18), trisomy 12 and deletion 13q. Subclonal abnormalities

and 25% of tumor cells in Case1, Case 2, and Case 3, respectively (**Figure 5**).

included an unbalanced t(1;8) which resulted in an extra copy of MYC (8q) and deletion of 3q (loss of BCL6). Case 2 and 3 did not demonstrate other genetic abnormalities.

Discussion

We report here 3 cases of B cell lymphoma with t(14;18)(q32;q21) translocation and an extra copy of c-MYC. Aberrant MYC overexpression is associated with uncontrolled cell growth and tumor metastasis [6]. Gain of MYC copy number has been reported associated with the elevated levels of mRNA and the corresponding MYC protein. FISH analysis of aggressive, mature B-cell neoplasm has shown that additional copy numbers of MYC, BCL2 or BCL6 could be demonstrated in diffuse large B cell lymphoma [8]. Other studies suggested that lymphomas with extra copy number of MYC or BCL2 were clinically aggressive and with poor prognosis comparable to lymphomas with rearrangements of the same loci [5]. These studies prompted the possibility that gain of MYC may play a similar role as MYC rearrangement. MYC copy number gain has been shown associated with poor disease-free survival and overall survival in lung adenocarcinoma [9]. However, the retrospective review of DHL cases demonstrated no significant association between survival time with the type of MYC alteration [10]. Moreover, a study of patients with DLBCL did not demonstrate correlation between the copy number of MYC and disease outcome [11]. Our experience suggested that although extra copy of c-MYC was associated with high Ki67 index and more extensive extranodal organ disease involvement, the overall clinical course and response to chemotherapy may correlate more with specific underlying disease (DLBCL vs. FL). Case 1 with morphology intermediate between DLBCL and BL was associated with very poor prognosis while the two FL demonstrated much better outcome, though they was associated with more aggressive morphology than regular FL. Complex genetic abnormality in addition to extra MYC and BCL2/IGH translocation in Case 1 may contribute to the aggressive morphology and poor clinical outcome as well.

The tumor cells demonstrated relatively higher Ki-67 labeling index in all three cases. Some studies demonstrated that Ki67 staining typically reflects the grade of follicular lymphoma, with high Ki67 labeling rate associated with worst disease outcome [12, 13]. In case 2, the diagnosis of high grade follicular lymphoma was supported by high Ki67 index. However, underlying cytogenetic can be more important predictor of disease outcome as compared to isolated Ki67 labeling index. Increased Ki67 index may be associated with various cytogenetic abnormalities, including c-MYC translocation or increased copy number with unknown clinical consequences. Complete remission in cases 2 and 3 may suggest that extra copy of c-MYC on the background of FL can be associated with better prognosis.

In summary, the results from our case study and literature review indicated that extra copy of c-MYC may possibly act similar to c-MYC translocation on the background of complex cytogenetic abnormalities, morphologically manifested as DLBCL. However, extra copy of c-MYC on the background of exclusive t(14;18) (q32;q21), manifested in follicular lymphoma can be associated with morphology and clinical outcome far from highly aggressive double hit/ gray zone lymphoma although more aggressive phenotype (higher Ki67 labeling index) and more extensive organ involvement were demonstrated as compared to low grade FL. Nevertheless, the clinical prognosis of such cases as compared to "classical" low grade FL remains to be elucidated.

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Disclosure of conflict of interest

None.

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