CYTOGENETIC INVESTIGATION OF ABDOMINAL LYMPH NODE CELLS OBTAINED BY LAPAROSCOPIC BIOPSY IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA

Summary. Aim: of this study was to evaluate the usefulness of tumor material obtained by laparoscopic diagnostic biopsy of the affected abdominal and retroperitoneal lymph nodes (LN) for cytogenetic analysis and to determine its diagnostic value. Methods: Laparoscopic (videosurgical) diagnostic biopsy was conducted in 32 patients using the original strategy developed in SI “IBPM of NAMS of Ukraine” aimed to provide easier access to affected LN and to prevent postoperative hemorrhage. The diagnosis of lymphoid neoplasm based on histopathological and immunophenotypic analyses of the biopsy tissue was established in 62.5% of patients. Cytogenetic investigations (karyotype analysis and FISH) of LN cells were performed in 11 patients with B-cell lymphoid neoplasm. Results: A wide spectrum of chromosomal aberrations was identified: in CLL/SLL – del(11)(q23); +8, +12; in FL – t(2;18;5)(р11;q21;q26); in DLBCL – add(14q); add(6)(p22). The following aberrations were revealed by FISH method: deletion of ATM gene in CLL/SLL, rearrangement of BCL2 and IGK genes in FL, amplification of IGH, BCL6, BCL2 genes in DLBCL. Conclusion: The expediency of laparoscopic biopsy in isolated abdominal LN impairment was proved for the timely diagnosis of lymphoid neoplasms.

INTRODUCTION

The Non-Hodgkin’s lymphomas (NHL) are the malignant neoplasms of lymphoid tissue, which comprise several lymphoproliferative disorders of different malignancy degree, character, grade of impairment, and peculiarities of clinical course. The primary mechanism of NHL is apparently explained by damage of genetic apparatus of lymphoid tissue caused by mutations, which leads to the abnormality of its cellular cycle regulation, disorders of differentiation and apoptosis as well as uncontrolled proliferation [3, 4].

The modern standard of NHL diagnostics includes characteristics of biological properties of tumor substrate on the basis of morphological, immunophenotypic analysis, genetic methods of examination, evaluation of clinical manifestations and dissemination of process with the help of instrumental methods (radiological, ultrasound investigation, computed tomography, etc.) [3, 9, 11]. The combination of these methods allows us to better understand the
pathogenesis and biological nature of tumors of lymphoid tissue, to divide them into the separate categories according to the WHO Classification of tumors of lymphoid tissue (2008) [26] and to individualize the treatment according to the prognostic risk factors.

The tumor material for the investigation is obtained by surgical method – by the excisional biopsy of tumor mass, mostly of lymph node (LN), inasmuch as the peripheral blood and the marrow in NHL case are often remained intact [8, 11, 19]. The needle biopsy is less informative in these patients, sometimes too complicated for accomplishment and dangerous; therefore it is accepted only in urgent cases [15, 28]. The surgical approach and subsequent biopsy of lymph mass become traumatic for patient in case of isolated impairment of thoracic LN, abdominal cavity LN or retroperitoneal LN in absence of peripheral lymphadenopathy. For the diagnosis of such isolated impairments the minimally invasive methods of videosurgery (laparoscopic diagnostics) were elaborated and introduced [14, 15, 18].

The standard diagnostics of the type of malignant lymphoma includes both the morphological (histological), immunophenotypic (immunohistochemical) analysis, and cytogenetic examination of malignant cells. Some types of NHL have specific diagnostic chromosomal aberrations (cytogenetic markers), others are detected by frequent chromosomal aberrations, which allow us to specify the type of NHL and to define its prognosis [5, 16, 22].

In modern cytogenetic diagnostics of NHL fully applied are two methods of analysis: conventional cytogenetics with differential staining of chromosomes, in which the whole karyotype is analyzed; and the method of fluorescent in situ hybridization (FISH), in which the targeted search for defined chromosomal abnormalities [3, 16] is conducted.

The aim of the research was to evaluate the usefulness of tumor material obtained by the laparoscopic diagnostic biopsy of isolated affected LN of the abdominal cavity and retroperitoneal area for the cytogenetic analysis and to determine its diagnostic value.

OBJECT AND METHODS

Within the period of 2007-2012 the laparoscopic biopsy of LN was performed in 32 patients due to isolated lymphadenopathy of abdominal cavity and retroperitoneal space without hyperplasia of peripheral LN in surgery clinic of SI “Institute of Blood Pathology and Transfusion Medicine of the National Academy of Medical Sciences of Ukraine”. No LN available for the biopsy were detected in two more cases during the diagnostic laparoscopy. In 31 patients the primary diagnostic interventions were performed, inasmuch as the reason of intraperitoneal lymphadenopathy (LAP) was unknown. In one patient with diagnosed chronic lymphocytic leukemia (CLL) the biopsy for the timely diagnostics of probable relapse (transformation) of neoplastic process was carried out. The patients gave their consent for using the biopsy material for research purposes.

The laparoscopic diagnostic biopsy was conducted by the original strategy developed at the institute aimed to provide easier access to the affected LN and to prevent postoperative hemorrhage (patent for an invention №87774) [8]. There were no postoperative complications. The obtained tissue was used for histological and when needed – immunohistochemical and cytogenetic analysis, which allowed to diagnose successfully 32 patients who underwent videosurgical diagnostic lymphadenectomy. The defined diagnoses were: CLL – confirmed in 1 patient, B-cell NHL (B-NHL) – in 14, T-cell lymphoma – in 1, Hodgkin’s lymphoma – in 4, tumor of pancreas with metastatic involvement of LN – in 4, gallbladder carcinoma with LN involvement – in 2, malignant neoplasm of ovary with secondary tumorous damage of greater omentum – in 2, extensive carcinomatosis of peritoneum – in 1, retroperitoneal fibroma – in 1, sarcoidosis of retroperitoneal LN – in 1 and tuberculosis of LN – in 1 patient. Thus, neoplasms of lymphoid tissue comprised 62.5% out of all cases of intraperitoneal LAP.

A laparoscopic biopsy material of 11 patients (5 females and 6 males aged from 50 to 90, median age – 70) with B-NHL diagnosed on the basis of the results of histological, immunophenotypic and cytogenetic methods was studied for determination of lymphoma type, prediction of disease course and treatment selection. The distribution of 11 patients
according to the type of B-NHL and some of their clinical data is given in table 1. According to this, cytogenetic tests were conducted in 4 patients with chronic lymphocytic leukemia/small lymphocytes lymphoma (CLL/SLL), 1 – with follicular lymphoma (FL), 1 – with marginal zone lymphoma (MZL), 5 – with diffuse large B-cell lymphoma (DLBCL).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Primary diagnosis</th>
<th>Final diagnosis</th>
<th>Treatment(number of chemotherapy courses)</th>
<th>Results of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>58/male</td>
<td>Progression/ transformation of CLL</td>
<td>CLL</td>
<td>Fludarabine + alemtuzumab (5); chlorambucil + methylprednisolone(4)</td>
<td>Resistance, progression, death</td>
</tr>
<tr>
<td>B</td>
<td>74/female</td>
<td>LAP/NHL?</td>
<td>CLL/SLL</td>
<td>Not conducted</td>
<td>Progression, death</td>
</tr>
<tr>
<td>C</td>
<td>60/male</td>
<td>LAP/ DLBCL?</td>
<td>SLL/CLL</td>
<td>Refusal</td>
<td>Progression, death</td>
</tr>
<tr>
<td>D</td>
<td>58/male</td>
<td>LAP, splenomegaly/ CLL?</td>
<td>CLL/SLL</td>
<td>Splenectomy; COP, CHOP</td>
<td>Partial remission</td>
</tr>
<tr>
<td>E</td>
<td>56/male</td>
<td>LAP/NHL?</td>
<td>FL</td>
<td>COP (5)</td>
<td>Partial remission</td>
</tr>
<tr>
<td>F</td>
<td>69/male</td>
<td>LAP, splenomegaly, bone marrow impairment</td>
<td>MZL of spleen</td>
<td>Splenectomy; fludarabine + cyclophosphamide (3)</td>
<td>Partial remission</td>
</tr>
<tr>
<td>G</td>
<td>74/male</td>
<td>LAP, splenomegaly</td>
<td>DLBCL</td>
<td>Not conducted</td>
<td>–</td>
</tr>
<tr>
<td>H</td>
<td>58/female</td>
<td>LAP, splenomegaly</td>
<td>DLBCL</td>
<td>CHOP (2), fludarabine (1)</td>
<td>Stable course</td>
</tr>
<tr>
<td>K</td>
<td>53/female</td>
<td>LAP, CNS impairment</td>
<td>DLBCL</td>
<td>CHOP</td>
<td>Progression, death</td>
</tr>
<tr>
<td>L</td>
<td>50/female</td>
<td>LAP</td>
<td>DLBCL</td>
<td>CHOP, etoposide</td>
<td>Stable course</td>
</tr>
<tr>
<td>M</td>
<td>90/female</td>
<td>LAP</td>
<td>DLBCL</td>
<td>Not conducted</td>
<td>–</td>
</tr>
</tbody>
</table>


Cytogenetic analysis of metaphases obtained using direct samples and long-term (72h) cell cultures of LN was conducted. We used standard procedure of *in vitro* cultivation of LN cells. Cells were prepared by standard procedure, which included the colchicine treatment, hypotonization, fixation and preparation of slides. The analysis of metaphase
chromosomes was conducted by G-banding with Wright’s stain. [2, 7, 24, 27]. The slides were analyzed with magnification of \(\times 1000\) by Olympus BX41 microscope («Olympus», Japan) at the Laboratory of Immunocytology and Genetics of Blood Malignancies of SI “IBPM of NAMS of Ukraine” using the chromosome analysis system CytoVision («Applied Imaging», Great Britain); and also at the Laboratory of Cytogenetics of Maria Skłodowska-Curie Institute of Oncology (Warsaw, Poland) by Axioskop 40 microscope («Carl Zeiss Jena», Germany) using karyotype analysis and FISH system («MetaSystems», Germany). The analysis and description of karyotype and FISH was performed according to the criteria of International System for Human Cytogenetic Nomenclature – ISCN, 2009 [20].

In some cases the metaphases were either not revealed or their quality was unsatisfactory. In some of such cases when the eligible for analysis metaphases were unavailable, the FISH method was used with proper probes: gene specific probe to \(\text{ATM}\) gene, BreakApart probes to the \(\text{c-MYC, BCL6, BCL2, IGK}\) genes, DualColor, DualFusion translocation probe to \(\text{BCL2}\) and \(\text{IGH}\) genes («Vysis», USA) as well as whole chromosome painting probes to chromosomes 2 and 5. All FISH tests were performed at the Laboratory of Cytogenetics of Maria Skłodowska-Curie Institute of Oncology. The preparation of specimens and procedure of hybridization were conducted according to D. Pinkel et al. [25] following recommendations of the probes manufacturers. The minimum of 200 interphase nuclei were analyzed.

RESULTS AND DISCUSSION

The indications for the laparoscopic anatomic and excisional biopsy were the hyperplasia of intraperitoneal and/or retroperitoneal LN in case of absence of enlarged peripheral LN along with lack of diagnostic findings revealed by general clinical, biochemical, special hematological tests, ultrasonic diagnostics, and computed and nuclear magnetic tomography. The diagnostic lymphadenectomy with open surgical approach by means of either laparotomy or thoracotomy is quite traumatic, accompanied by long postoperative period, and, which is more important, delaying the administration of required treatment until complete healing of postoperative wound. The application of videosurgical methods for diagnostics of isolated LAP provides significant positive effect: minimal surgical trauma, short period of operation, fast recovery of the patient and, of course, opportunity to prescribe adequate disease specific therapy directly after verification of the diagnosis [14, 15, 18]. However, such comparatively less traumatic procedure is often accompanied by technical complications, increased bleeding, anemia, may also involve intraoperative bleeding, which leads to failure and requires conversion in 17% of cases [13].

Cytogenetic investigation of cells prepared from laparoscopically obtained LN was conducted in 11 patients with B-NHL. In 6 (55%) of the cases the adequate for conventional karyotyping metaphases were obtained, whereas in other 6 patients FISH was performed. In 3 of these patients both two methods were simultaneously applied. In the remaining 2 cases no mitoses were obtained and FISH method was also not applied. The results of cytogenetic tests are shown in table 2.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>FISH</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>46,XY,del(11)(q23)[15] nuc ish (ATM=1)[204/235]</td>
<td>CLL/SLL</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>No metaphases obtained nuc ish (MYC=2)[291/291] nucish (BCL6=2)[233/233]</td>
<td>SLL/CLL</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>No metaphases obtained</td>
<td>Not performed</td>
<td>CLL/SLL</td>
</tr>
<tr>
<td>E</td>
<td>46,XY,t(2;18;5)(p11;q21;q21)[20]/46,XY[1] nuc ish (BCL2=2)(5'BCL2 sep 3'BCL2=1)[194/261] nuc ish (5'IGK=3,3'IGK=2)(5'IGK con 3'IGK=2)[16]/(IGK=2)[10] 46,XY,t(2;18;5)(p11;q21; q21); ish t(2;18;5)(wcp5+,wcp2+; wcp5+,wcp2+; wcp2+,wcp5-)[4]</td>
<td>FL</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>No metaphases obtained</td>
<td>Not performed</td>
<td>MZL</td>
</tr>
<tr>
<td>G</td>
<td>46,XY.add(14q)[19] nuc ish (BCL2=2,IGH=3)[157/209 ] nuc ish (5'BCL6+2,3'BCL6=3) (5'BCL6 con 3'BCL6=2)[165/246]</td>
<td>DLBCL</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>46,XX.add(6)[p22][14]/46,X X[4]</td>
<td>Not performed</td>
<td>DLBCL</td>
</tr>
<tr>
<td>K</td>
<td>46,XX[20]</td>
<td>Not performed</td>
<td>DLBCL</td>
</tr>
<tr>
<td>L</td>
<td>No metaphases obtained nuc ish (IGH=2,BCL2=2~10)[208 /208]</td>
<td>DLBCL</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>No metaphases obtained nuc ish (MYC=2)[222/222] nuc ish (BCL6=2)[219/219]</td>
<td>DLBCL</td>
<td></td>
</tr>
</tbody>
</table>

The metaphases were obtained in 2 of the patients with CLL/SLL, in other 2 – mitoses were not detected, due to low mitotic activity of the cells (see table 1). In patient A with fast enlargement of abdominal cavity LN after a good response of CLL to fludarabine treatment the deletion of long arm of chromosome 11, del(11q), was found in LN cells (Fig. 1). Due to bad quality of chromosomes, which often happens in patients with lymphoid neoplasms, the breakpoint in del(11q) was not precisely defined. For this reason the FISH test was conducted with using the gene specific probe to \(ATM\) gene (locus 11q23). In 87% of the cells only 1 signal from \(ATM\) gene was detected, which was the evidence of its deletion (Fig. 2). This result confirmed conclusion of morphological (small cell NHL with diffuse growth pattern) and immunohistochemical (CD5’CD19’CD23’, CD38’>30%) analyses – enlargement of LN of abdominal cavity in this patient was caused by progressing of CLL with unfavorable prognostic features. Later this patient proved to be strongly resistant to cytostatic therapy: 5 cycles of fludarabine + alemtuzumab...
combination therapy and 4 cycles of chlorambucil + methylprednisolone treatment induced no response, and the patient subsequently died from disease progression.

Fig. 1. Patient A. Karyotype of LN cells: 46,XY,del(11q)

Fig. 2. Patient A. The result of FISH analysis with ATM gene specific probe. Presence of one red signal shows presence of del(11)(q23).

In LN cells of patient B complex chromosomal abnormalities were detected involving chromosomes 12 and 8 (Fig. 3), which corresponded to the morphological diagnosis of CLL/SLL. This female patient was diagnosed on terminal stage of disease and shortly died; treatment was not conducted.
In patient C with isolated LAP and suspicion of DLBCL the results of morphological and cytogenetic analysis corresponded. Histologically – lymphoma of mature lymphocytes; mitoses were not revealed neither in direct sample nor in the culture of lymph node cells. FISH analysis performed on interphase nuclei using BreakApart probes for BCL6 (locus 3q27) and c-MYC (locus 8q24) genes detected two fusion signals in all cells, which confirmed the absence of rearrangement of these genes (Fig. 4). As to the disease course, leukemia of lymphoma developed with occurrence of CD5⁺CD19⁺CD23⁺ lymphocytes in peripheral blood.
In a sample of patient D the metaphases were not detected and FISH analysis was not conducted. On the basis of morphological and immunochemical tests the CLL/SLL was diagnosed. Treatment (splenectomy, cycles of COP, CHOP) resulted in partial remission.

In patient E, whose histological features of LN biopsy corresponded with FL, the complex translocation involving chromosomes 2, 5 and 18 was detected (see table 1, Fig. 5). The abnormality is quite unusual, but the breakpoint is representative for typical FL with t(14;18)(q32;q21), which corresponds to morphological diagnosis. Several FISH analyses were conducted in order to determine the type of translocation using BreakApart probes for IGK (locus 2p11) and BCL2 (locus 18q21) genes as well as whole chromosome painting probes to chromosomes 2 and 5. The fragment of chromosome 5 was detected on derivative chromosome 2, fragment of chromosome 2 – on derivative chromosome 18, fragment of chromosome 18 – on derivative chromosome 5 (Fig. 6). The investigation using BreakApart probes to IGK gene detected two co-localized signals from 5’ and 3’ fragments and additional signal from 5’ fragment, which was an evidence of rearrangement of this gene. During FISH analysis with BreakApart probe to BCL2 gene the separation of one of the signals was detected, which is suggestive of BCL2 gene rearrangement. Thus, the presence of complex translocation t(2;18;5)(p11;q21;q21) was detected. This patient subsequently had a good response to 5 cycles of COP.

Fig. 5. Patient E. Karyotype of LN cells: 46,XY,t(2;18;5)(p11;q21;q21)
Fig. 6. Patient E. The result of FISH analysis with whole chromosome painting probes to chromosomes 2 (red fluorochrome) and 5 (green fluorochrome). The fragment of chromosome 5 on derivative chromosome 2 and fragment of chromosome 2 on derivative chromosome 18 was detected.

As a result of examination of patient F with intraperitoneal LAP, significant splenomegaly and presence of approximately 5% of atypical lymphoid cells in bone marrow, MZL of spleen was diagnosed on the basis of histological investigation of intraperitoneal LN with CD5 cyCD20’CD30’ tumor cells immunophenotype. No metaphases were detected in the samples of this patient probably due to low mitotic activity of the cells; FISH was not conducted. The patient was splenectomized and underwent 3 cycles of chemotherapy following the FC scheme (fludarabine + cyclophosphamide), which resulted in achieving remission.

The metaphases, adequate for further analysis, were detected in samples of the 3 out of 5 patients with morphologically confirmed diagnosis of DLBCL (see table 1). In patient G the extra-material on the long arm of chromosome 14 was detected, the partner of translocation was impossible to define. In this patient with DLBCL with add(14q) FISH analysis was conducted with BreakApart probes to BCL6 gene and translocation probe to IGH (locus 14q32) and BCL2 (locus 18q21) genes in order to detect translocation t(14;18)(q32;q21). As a result we revealed the increased number of copies (in groups of 3 copies) of IGH gene in 75% of cells (Fig. 7) and fragment 3’ of BCL6 gene – in 67% of cells, without, however, BCL6 gene disruption (Fig. 8). Fusion signals from IGH and BCL2 genes were not detected, which suggested the absence of t(14;18).
**Fig. 7. Patient G.** Result of FISH analysis with translocation probe to *IGH* and *BCL2* genes. The typical for translocation t(14;18) fusion signal was not detected, but the presence of extra-copy of *IGH* gene (extra green signal) was detected in the investigated cells.

**Fig. 8. Patient G.** Result of FISH analysis with *BCL6* gene BreakApart probe. Presence of two fusion signals and one extra red signal suggest the absence of rearrangement of *BCL6* gene and presence of extra copy of fragment 3’ of this gene.

In patient H 14 metaphases with an extra material of unidentified origin on chromosome 6 were detected and 4 metaphases – with otherwise normal female karyotype (Fig. 9); FISH analysis was not conducted. The patient got 2 cycles of CHOP and 1 cycle of fludarabine, without significant response.

In a sample of patient K the normal female karyotype was detected without cytogenetically visible abnormalities, which, according to the published research data, is the evidence of the stable course of disease. However, according to the clinical signs disease progression was established in this patient despite the treatment performed (2 cycles of CHOP). This could probably occur due to the gene modifications, which are impossible to define by the standard cytogenetic method. FISH analysis in this case was not conducted.
Fig. 9. Patient H. Karyotype of LN cells: 46,XX,add(6)(p22)

No metaphases were detected in the material of the LN of 2 patients with DLBCL due to low mitotic activity of the cells. The FISH analysis was subsequently conducted in these patients. In case of patient L, the translocation probe of IGH and BCL2 genes was applied. There were no fusion signals from these genes, which evidenced the absence of t(14;18), however, the increase of number of copies (from 3 to 10) of BCL2 gene was detected in all the cells (Fig. 10). The patient underwent CHOP and CHOP + etoposide chemotherapy without prominent clinical effect.

Fig. 10. Patient L. Result of FISH analysis with translocation probe to IGH ta BCL2 genes. The typical for translocation t(14;18) fusion signal was not revealed, but the presence from 3 to 10 extra copies of BCL2 gene (extra red signals) was detected in the analyzed cells.
The BreakApart probes to $BCL6$ and $c-MYC$ genes were applied on interphase nuclei of patient $M$. In this case two unseparated signals were revealed in each cell, which is suggestive of the absence of any abnormalities in these genes.

Presently there is no typical cytogenetic marker defined for CLL, however, there exist certain aberrations frequently involved, such as del(13)(q14), del(11)(q23), del(17)(p13), trisomy of chromosome 12 [1, 3, 10]. The prognostic significance of these abnormalities was studied in available literature sources. The deletion del(11)(q23) is among the factors of intermediate prognosis [16]. It is known that locus 11q23 contains $ATM$ gene and its deletion may result in development of chromosome instability syndrome; in case of loss of $ATM$ gene the course of CLL acquires an adverse pattern [3, 10, 23]. In presence of this aberration the resistance to cytostatic treatment or short-term treatment response is often observed. In case of patient $A$ with this abnormality both unfavorable disease course (progression with massive intraperitoneal LAP) and absence of response to intensive chemotherapy were stated. Some of the research papers and reports suggest that it is tumorous form of CLL that is most often characterized by deletion of $ATM$ gene. The deletion of chromosome 11 is accompanied by specific profile of expression of adhesion receptors on leukemic lymphocytes, which may explain their accumulation in LN with minor peripheral blood lymphocytosis.

There are contradictory views concerning the prognostic significance of trisomy of chromosome 12 in CLL/SLL. According to some reports, the stable course of disease is quite typical for this aberration; according to the others it is rather prone to disease progression [1]. In CLL patients with rapid disease progression the following aberrations are often found: deletion del(17)(p13), trisomy of chromosome 12 or duplication of its long arm and complex abnormalities. Our observations (patient $B$) suggest that the trisomy of chromosome 12 is a prognostically unfavorable factor in CLL disease course, especially if atypical morphology of malignant lymphocytes is present. On the contrary the del(13)(q14) deletion, in those cases where it is the only abnormality, shows the favorable course of disease[3, 4, 12].

Typical abnormality for the FL is t(14;18)(q32;q21) translocation [3]. The key point of the development of this lymphoma is transmission of $BCL2$ gene from chromosome 18 to chromosome 14, 2 or 22 (t(14;18)(q32;q21), t(2;18)(p12;q21) or t(18;22)(q21;q11)). As a result, $BCL2$ gene is influenced by genes of immunoglobulin light and heavy chains. The stimulation of $BCL2$ results in hypoppression of the antiapoptotic $BCL2$ protein leading to suppression of apoptosis [3, 17]. In rare cases of FL and DLBCL the t(5;18)(p11;q21) translocation, in which $BCL2$ gene is also involved, may also occur [3]. In the complex translocation t(2;18;5)(p11;q21;q21) of patient $E$ involved were $IGK$ (locus 2q11) and $BCL2$ (locus 18q21) genes, and this rearrangement was confirmed by FISH analysis.

DLBCL does not have a specific genetic marker described. For this heterogenic in terms of its clinical, morphological, immunological and genetic signs, group of mature lymphoid neoplasms the series of genetic abnormalities were described, including rearrangement of genes of immunoglobulin light and heavy chains ($IGL$, $IGH$), and somatic mutations of their variable regions, modifications of $BCL2$ (locus 18q21), $BCL6$ (3q27), $TP53$ (17p13), $c-MYC$ (8q24), $FAS$ (10q24) genes [3, 6, 17, 22, 23]. There were attempts made by some researchers to connect cytogenetic aberrations with morphological subtypes (centroblast-like, immunoblast-like, T-cell/histiocytes-rich etc.) and variants of DLBCL according to gene expression profile (germinai center B-cell – GCB, activated B-cell – ABC), but mostly unsuccessful. Somewhat more is known about prognostic significance of genetic aberrations. According to this, the most frequent abnormality, t(14;18)(q32;q21), resulting in formation of chimeric $IGH/BCL2$ gene is typical for FL, but occurs also in 20-30% of patients with centroblast-like DLBCL, significantly worsening the prognosis of this subtype [3, 17, 22]. So far there are no precise criteria, except for subjective morphological signs, which would allow us to differentiate DLBCL possessing this translocation from stage III FL with transformation into DLBCL. It is unknown, if these two variants are different prognostically [3, 17]. Adverse course of DLBCL is associated with the high numbers of $BCL2$ gene copies [22, 23]. We didn’t define t(14;18) in examined DLBCL patients, but the presence of $BCL2$ gene amplification in patient $L$ was accompanied by resistance to chemotherapy.
Among some other prognostically unfavorable signs of DLBCL there are hyperexpression of protein p53 caused by abnormalities in TP53 gene. The abnormalities involving 8q24 (c-MYC) are rare in DLBCL and are the sign of new entity, first time defined in the WHO classification in 2008 as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma; they are the evidence of the very aggressive course of disease [3, 5, 17, 21].

It is reported in scientific sources that in around 20-40% of DLBCL patients BCL6 gene is involved in t(3;14)(q27;q32) and some other translocations, and high level of BCL6 protein expression is connected with a good prognosis of DLBCL [3, 12]. In patient G amplification of IGH gene and of BCL6 gene 3’ fragment without disruption of BCL6 gene were detected. Since the translocation probe to BCL6 and IGH genes was not applied in this case, it could not be stated precisely that presence of an extra material on 14 chromosome, add(14q), was not caused by t(3;14). Regrouping of gene segments in locus IGH (14q32) may provoke the formation of genes, coding specific heavy chains in B-cell lymphomas [3, 6, 17]. The prognostic significance of genetic abnormalities in patient G couldn’t be defined, since after the laparoscopic surgery he was lost to follow-up.

The abnormalities of chromosome 6 defined in patient H are typical for myelo- and lymphoproliferative disorders. The aberrations in this chromosome in NHL have no diagnostic or prognostic significance and confirm only clonal proliferation [17].

In many cases of DLBCL there are complex cytogenetic abnormalities. There is a hypothesis in some research papers that involving of many chromosomes in cytogenetic aberrations is typical for unfavorable course of the neoplasm [22, 23].

The 2008 WHO classification emphasizes the importance of prognostic significance of DLBCL stratification according to gene expression profile (approximately 12000 of genes), which is defined by the latest genome technologies on the basis of DNA and oligonucleotide microarray [12]. It allows us to define precisely the GCB-like subtype of DLBCL with a good prognosis and postgerminal ABC-like subtype with an adverse prognosis. Nevertheless, this division is not introduced in classification of lymphomas and practice yet, because gene expression profiling has not yet become a routinely available diagnostic tool, and immunohistochemical surrogate markers (CD10 and/or BCL6 expression in GCB and MUM1/IRF4, Cyclin D in ABC) correlate poorly with genome investigations [21].

The use of conventional cytogenetics in a majority of mature-cell B-NHL is not always successful due to low mitotic activity of the cells or bad quality of metaphases [16, 23]. Conventional karyotyping enables detection of both marker (specific) diagnostic chromosomal abnormalities and variant abnormalities, which are mainly the evidence of unfavorable course of disease [16, 22]. The advantage of FISH is a possibility to analyze the interphase nuclei, as well as to identify the complicated chromosomal abnormalities and reveal small (submicroscopic) abnormalities, which cannot be seen by conventional cytogenetics. In addition, in those cases when fresh bioptic tissue is unavailable, FISH may be conducted on cytogenetic specimens and histological sections from the archive paraffin embedded tissue [3, 22, 25].

CONCLUSIONS
1. In those cases when only isolated abdominal and retroperitoneal LN are affected it is recommended to conduct videosurgical (laparoscopic) diagnostic biopsy which is less traumatic, safe procedure allowing to diagnose lymphoid neoplasm in 62,5% of isolated LAP.
2. Diagnostic laparoscopy gives opportunity to conduct the standard for NHL morphological, immunohistochemical and, in particular, cytogenetic tests on obtained bioptic material in order to determine proliferation type, prognosis of disease course and for treatment selection.
3. As a result of conducted cytogenetic tests (karyotyping and FISH) of LN cells in patients with B-cell lymphoid neoplasm the number of chromosomal aberrations were detected: del(11)(q23); +8, +12 – in CLL/SLL;
t(2;18)(p11;q21;q21) – in FL; add(14q); add(6)(p22) – in DLBCL. FISH tests enabled us to detect the deletion of
ATM gene in CLL/SLL, rearrangement of BCL2 and IGK genes in FL, amplification of IGH, BCL6, BCL2 genes –
in DLBCL.
4. The results of cytogenetic tests obtained during the diagnostic laparoscopy of LN have important clinical
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