Double Hit Lymphoma (DHL)
Triple Hit Lymphoma (THL)

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Published in Atlas Database: April 2017
Online updated version: http://AtlasGeneticsOncology.org/Anomalies/DoubleTripleHitNHLID1613.html
DOI: 10.4267/2042/68886

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Abstract

The role of cytogenetics in identification of double-hit lymphoma, a subset of a high grade large B-cell lymphoma.

KEYWORDS
Diffuse large B-cell lymphoma; double-hit lymphoma; triple-hit lymphoma; MYC; BCL2; BCL6.

Identity

Double Hit Lymphoma (DHL)
Triple Hit Lymphoma (THL)

Note
The "double-hit" lymphoma is a genotype feature of lymphoma that exhibits translocations of both the MYC /8q24 gene and a second site, most often the BCL2/18q21 or less commonly the BCL6/3q27 gene. A rare "triple-hit" lymphoma harboring MYC, BCL2, and BCL6 translocations has also been identified. Cytogenetics is necessary in establishing the diagnosis of these lymphomas as they tend to manifest an aggressive behavior, and poor outcome when treated with standard therapy.

Clinics and pathology

Disease
Double-hit lymphoma (DHL), triple-hit lymphoma (THL), diffuse large B-cell lymphoma (DLBCL), high grade B-cell lymphoma (HGBL) with MYC, BCL2 and/or BCL6 rearrangements

Epidemiology
The DH lymphoma most commonly affects elderly patients with a median age of 62-74 years (range 18-96 years), and both sexes are affected with a slight male predominance. The incidence of DH genotype has been difficult to assess because fluorescence in situ hybridization (FISH) studies have not been used widely on unselected series, and the published data may be biased toward a specific type of lymphoma. Nevertheless, DH lymphoma has been reported in 5-15% of patients with DLBCL and aggressive B-cell lymphoma. Comprehensive analysis of Mitelman Database of Chromosome Aberrations in Cancer (Aukema et al, 2011, Oliveira et al 2017), DH lymphomas with breaks at MYC, BCL2 and/or BCL6 regions were identified in 14% of DLBCL cases; 62% of those involved MYC and BCL2 sites, 18% involved MYC and BCL6 while the remaining cases had triple translocations. Using FISH, Barrans et al (2010) identified DH and TH genotypes in 12% cases with de novo DLBCL; the majority of these
cases had BCL2 and MYC translocations (66%), BCL6 and MYC breaks in 10% and breaks in all three sites in 24% of cases. MYC and BCL2 translocations are also found in a small proportion of follicular lymphoma. We identified MYC breaks in 7% of t(14;18)-bearing follicular lymphoma cases; all described to have a high grade morphology with Burkitt-like or blastic morphology replacing residual follicles (Mohamed et al, 2001). Pedersen et al have reported that in transformed lymphoma, DH genotype was very frequent found in 21% of cases; all had follicular lymphomas (Pedersen et al 2012).

**Clinics**

Patients with DH lymphomas commonly have an aggressive clinical course, characterized by advanced stage disease, extranodal involvement, and high serum lactate dehydrogenase levels. The extranodal sites include the bone marrow, central nervous system, gastrointestinal tract, liver, spleen, lung, pleural fluid, testis, ovaries, skin, and thyroid gland. Bone marrow is involved in about 50% of patients and many have a leukemic disease. The International Prognostic Index score (IPI score) is often intermediate-high or high (Barrans et al 2010, Petrich et al 2014). Most patients have a de novo disease while a minority (10%) present with a history of a low-grade follicular lymphoma or other B-cell lymphoma, then develop DHL presumably by the acquisition of MYC rearrangement.

**Double Expresser lymphoma**

Immunohistochemistry (IHC) has identified a fraction of DLBCL cases with high expression of MYC and BCL2 proteins (Perry et al 2014, Mahmoud et al 2015). These double expresser lymphomas (DE) are lacking MYC and BCL2 translocations and are usually of non-GC types. DE lymphomas are much more common than DH lymphomas, with approximately 30% of DLBCL being reported as DE lymphomas. Most studies use a cutoff of 40% for MYC IHC and a cutoff of 70% for BCL2 to define DE lymphoma (Green et al 2012 A). Clinically, the DE lymphomas have more aggressive clinical course than those lacking MYC and BCL2 protein expression.

**Pathology**

Because DHL is genotypically defined; it is not restricted to a specific histology subtype. Most tumors are medium to large in size, with atypical morphology and very high proliferation rate. Previously, DH lymphomas were diagnosed as diffuse large B-cell lymphoma (DLBCL), aggressive B-cell lymphoma not otherwise specified (NOS) or Burkitt-like lymphoma. Rare cases were classified as lymphoblastic lymphoma. The increase recognition of these lymphomas led the 2008 WHO classification of hematopoietic and lymphoid neoplasms to introduce a provisional category of “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL”, (BCLU) to recognize a subset of very aggressive lymphoma in which the distinction between DLBCL and BL is difficult (Campo et al 2011, Swerdlow et al 2014). The 2008 WHO criteria were somewhat subjective and vaguely defined; as a result, recent studies have not been consistent with survival and disease outcome. Therefore, separation of these cases has become necessary to better define these clinically aggressive tumors. In the revised 2016 WHO classification of lymphoid neoplasms, all LBCL with "double hit" and "triple hit" genotype are included in a single category of “high-grade B-cell lymphomas (HGBL) with MYC and BCL2 and/or BCL6 rearrangements” (Swerdlow et al, 2016).

**Immunophenotype**

Most double hit lymphomas have a germinal center (GC) phenotype with expression of CD10 and BCL6 and lack of MUM1/IRF4. Expression of BCL2 protein is detected in almost 95% of cases supporting the observation that the BCL2 translocation is mainly found in GC type of DLBCL, and also MYC translocations are associated with a GC molecular profile in DLBCL. The proliferation index as measured by Ki67 immunohistochemical staining is often high (>90%). Therefore, although not very specific, co-expression of CD10, BCL6, BCL2, and a high Ki67 may predict double hit genotype in tumors morphologically diagnosed as DLBCL (Aukema et al 2011, Swerdlow et al 2014).

**Treatment**

Standard therapies such as R-CHOP developed for patients with DLBCL are less effective in DHL, with a median overall survival of 12 months or less, as the majority of patients experience disease progression. Currently, more, intensive chemotherapy such as dose adjusted R-EPOCH therapy, is being evaluated which appears to be effective in many DH lymphoma patients. Consideration for frontline stem cell transplant in responding patients are also suggested (Okí et al 2014, Petrich et al 2014, Dunleavy 2015).

**Prognosis**

Patients with classic, FISH-defined DH lymphomas have a very poor prognosis, usually present with high clinical risk factors, such as high IPI scores and bone marrow involvement. The outcome is dismal with rare long term survival after standard therapy (Perry et al 2014, Petrich et al 2014, Okí et al 2014). Several recent studies have indicated that the
outcome of DE lymphomas, as defined by IHC is inferior to that of DLBCL but better than that of DH lymphomas. Therefore, the co-expression of MYC and BCL2 proteins in DLBCL is considered a prognostic biomarker of an inferior outcome (Green et al, 2012 B).

Cytogenetics

Double-hit lymphomas are usually seen in the context of a complex karyotype containing multiple numerical and structural aberrations (Figure 1). Over 2/3 of cases demonstrate dual translocations involving 8q24/MYC and t(14;18)/BCL2 breakpoints. Less commonly the translocations implicate 8q24/MYC and 3q27/BCL6 sites and rare cases have breaks in all three sites, referred to a "triple hit" lymphoma. The partner gene(s) involved in MYC/8q24 translocations are the IG genes in about 60% of DHL cases; more frequently affecting the IG light chain loci 22q12/IG lambda and 2p12/IG kappa loci than the heavy chain 14q32/IGH (Aukema et al 2011, Swerdlow et al 2014). Non-IG partners are found in 40%, recurrently involved 19p13, 1q24, 9p13, or non-identified partner regions (Figure 1). Deletions of chromosome 17p13/TP53 are present in 20-30% of cases, which can further induce genomic instability, and contribute to the aggressive course of the disease (Figure 1).

Conventional cytogenetics is not always feasible since fresh tissue is required, and small translocations can be overlooked in a complex karyotype. Therefore, most cases currently are detected by FISH using labeled probes targeting genes of interest. FISH studies can be successfully performed with high reproducibility in almost all cases using formalin-fixed paraffin-embedded tissue sections, although smears, touch imprints, or pellet of metaphase and interphase cells can also be used. Today, most clinical cytogenetic laboratories are using a panel of FISH probes for detection of double hit lymphomas including LSI MYC break-apart (BA), LSI BCL6 BA, and LSI IGH-BCL2 dual fusion translocation probes. The IGH-MYC dual fusion translocation probe is useful in picking up occasional cases missed by the MYC BA probe and also in identifying the MYC partner in a subset of the cases (Figure 2 B). Collectively, FISH is considered the "Gold-Standard" test in identifying DH and TH lymphomas. At present it is a subject of debate, if all DLBCL should have FISH testing to detect MYC, BCL2, and BCL6 rearrangements or should FISH only performed on selected cases with high grade morphology and CG phenotypes. Guidelines are warranted to avoid high cost and at the same time not miss those aggressive lymphomas (Pedersen et al 2012, Sesques and Johnson 2017).
Double Hit Lymphoma (DHL): Triple Hit Lymphoma (THL)

Figure 2: FISH panel for "double-hit" lymphoma, A; MYC BA probe set showing a split/translocation pattern. B; IGH/MYC,CEP8 tricolor dual fusion translocation probe set showing two fusion signals indicating the presence of t(8;14). C; IGH/BCL2 dual fusion translocation probe set showing two fusion signals indicating t(14;18). D; BCL6 BA probe set showing a split/translocation pattern (all probes purchased from Abbott Molecular Inc, Des Plaines, IL USA).

Genes involved and proteins

**MYC v-myc myelocytomatosis viral oncogene homolog (avian)**

**Location**
8q24.21

**Note**
Alternative symbols: MYC proto-oncogene, bHLH transcription factor, v-myc avian myelocytomatosis viral oncogene homolog.

**Protein**
The product of the MYC gene has a molecular mass of 65 kD, is located predominantly in the nucleus, and binds to DNA. It is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, metabolism, DNA repair, apoptosis and cellular transformation. MYC operates as a transcription factor, being involved in almost every aspect of cell biology. It has been reported to regulate the expression of almost 15% of genes within the human genome. Over expression of MYC is associated with uncontrolled cell growth and metastasis whereas loss of its expression delays growth and promotes differentiation. Some of the most biologically important MYC target genes are cyclins and cyclin-dependent kinases (CDKs), resulting in accelerated cell cycling. Additionally, MYC is involved in the regulation of many microRNA (miRNA) expressions, including both the tumor suppressor and the oncogenic miRNAs (Aukema et al 2011, Sesques et al 2017). MYC induces DNA stress and activates the TP53 checkpoint, leading to apoptosis (Hoffman and Liebermann 2008).

**BCL2 (B-cell leukemia/lymphoma 2)**

**Location**
18q21.33

**Note**
Alternative symbol: Apoptosis regulator; B-cell leukemia/lymphoma 2.

**Protein**
BCL2 protein is the most potent member of a large BCL2 family which regulate cell death (apoptosis), by either inducing pro-apoptotic proteins or inhibiting apoptosis. BCL2 is widely expressed in immature B cells and memory B cells but is down-regulated in GC B cells, partially because of repression by BCL6. It is localized to the outer membrane of mitochondria, where it plays an important role in promoting cellular survival and inhibiting the actions of pro-apoptotic proteins. The pro-apoptotic proteins in the BCL-2 family, including BAX and BAK1, normally act on the mitochondrial membrane to promote permeabilization and release of cytochrome C and ROS1 that are important signals in the apoptosis cascade. These pro-apoptotic proteins are inhibited by the function of BCL2.

**BCL6 (B-Cell Lymphoma 6)**

**Location**
3q27.3

**Note**
Alternative symbols: B-cell lymphoma 6; Zinc finger protein 51 (ZNF51).

**Protein**
The protein encoded by this gene is a zinc finger transcription factor and contains an N-terminal POZ domain. This protein acts as a sequence-specific repressor of transcription, and has been shown to modulate the transcription of STAT-dependent IL-4 responses of B cells. It can interact with a variety of POZ-containing proteins that function as transcription co-repressors. BCL6 functions as a transcriptional repressor of many target genes involved in apoptosis, DNA damage response, cell cycle control, proliferation, and differentiation. These genes are BCL2, TP53, IRF4, and PRDM1 (BLIMP1), the latter being essential for maturation into plasma cells. As a result of the BCL6-mediated repression of TP53, somatic hypermutation and class switch recombination are facilitated. Approximately...
30% of diffuse large cell lymphomas and 10% of follicular lymphomas have 3q27 chromosomal translocations that upregulate expression of BCL6 by juxtaposing to IG or non-IG promoters to the BCL6 coding domain. BCL6 is widely expressed in many tissues, but in B-cells it is mostly restricted to germinal center B cells. The expression of BCL6 can be demonstrated in tissue sections by IHC. It is present in both healthy and neoplastic germinal centers. It therefore demonstrates both reactive hyperplasia in lymph nodes and a range of lymphomas derived from follicular B-cells, such as Burkitt's lymphoma, follicular lymphoma, and the nodular lymphocyte predominant subtype of Hodgkin’s disease. It is often used together with antibodies to Bcl-2 antigen to distinguish neoplastic follicles from those found in benign hyperplasia, for which Bel-2 is negative.

**Result of the chromosomal anomaly**

**Fusion protein**

**Note**

MYC translocations, a biological hallmark of BL, can also be detected in other B-cell lymphomas DLBCL, BCLU, and FL but at lower frequencies (mohamed et al 2001). However, there are some fundamental differences between MYC translocation in BL and in other mature B-cell lymphomas. In BL the MYC translocation always involves one of the IG genes, (IGH, IGL or IGK) and is considered a disease-initiating event which occurs in the context of a rather simple karyotype (Dave et al 2006). In contrast, MYC translocations in other mature B-cell lymphomas frequently involve non-IG partners and are mostly found in complex karyotypes, often in addition to well-known primary aberrations such as t(14;18) or t(3;14) translocation. In these lymphomas, MYC translocation is considered as a secondary event occurs during disease progression. Dave et al reported that the gene expression of MYC translocations in BL tend to be simple and control several genes involved in proliferation. In contrast, MYC aberrations in DLBCL-targeted genes are associated with nuclear factor KB (NF-kB) signaling and antiapoptotic cascades. Other mechanisms of MYC deregulation in aggressive B-cell lymphomas are mutations, increase copy number and transcriptional upregulation, mainly by B-cell receptor and NF-kB signaling. Recent studies show that microRNAs may upregulate MYC expression. In clinical setting, MYC aberrations can be detected by conventional cytogenetics, FISH, and recently by IHC.

In t(14;18) follicular lymphoma, the transcription of BCL2 is "constitutively" upregulated that leads to a survival advantage of the involved B cells. BCL2 overexpression in B cells also leads to impaired DNA repair by blocking nonhomologous end-joining activities of Ku proteins essential for repair of both RAG1 / RAG2 - and AICDA-mediated breakpoints. The t(14;18) is thought to occur early in B-cell development however, it is insufficient to cause follicular lymphoma because t(14;18) is rarely observed as a sole karyotypic abnormality (Mohamed et al 2001) and also can be found in mature B cells in healthy individuals. The overexpression of BCL2 makes the cells susceptible for additional genomic alterations such as MYC or BCL6 mutation and rearrangement. BCL2 protein is detected in over 50% of DLBCL and in 75% of HGBL, but is not expressed in BL.

**References**


Dunleavy K. Aggressive B cell Lymphoma: Optimal Therapy for MYC-positive, Double-Hit, and Triple-Hit DLBCL. Curr Treat Options Oncol. 2015 Dec;16(12):58


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Mohamed AN, Palutke M, Eisenberg L, Al-Katib A. Chromosomal analyses of 52 cases of follicular lymphoma with t(14;18), including blastic/blastoid variant. Cancer Genet Cytogenet. 2001 Apr 1;126(1):45-51


Sesques P, Johnson NA. Approach to the diagnosis and treatment of high-grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangements. Blood. 2017 Jan 19;129(3):280-288


This article should be referenced as such:

Mohamed AN. Double Hit Lymphoma (DHL); Triple Hit Lymphoma (THL) Atlas Genet Cytogenet Oncol Haematol. 2018; 22(4):142-147.