Author's response to reviews

**Title:** Clear cell variant of diffuse large B-cell lymphoma: a case report

**Authors:**

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**Version:** 5  **Date:** 8 December 2010

**Author's response to reviews:**

Reviewer: Lutfi Alia

**GENERAL CONSIDERATIONS**

1. INTRODUCTION

Line 4 - 9. The authors should provide reference(s) supporting this statement.
Line 21 - 25. The authors should provide reference(s) supporting this argument.
Line 28 - 30. The authors should provide reference(s) supporting this statement.

New revised version:

Diffuse large B-cell lymphoma (DLBCL) is the commonest type of lymphoid tumor world-wide. This category was included both in the REAL and WHO Classification aiming to lump together all malignant lymphomas characterized by the large size of the neoplastic cells, B-cell derivation, aggressive clinical presentation, and the need for highly effective chemotherapy regimens. (2)

These tumors are detected as primary or secondary forms both at the nodal and extra nodal levels, in immunocompetent hosts as well as in patients with different types of immunosuppression. They display a significant variability in terms of cell morphology and clinical findings, which justifies the identification of variants and subtypes. (2)

Diffuse large B-cell lymphoma (DLBCL) is a diffuse proliferation of large neoplastic B lymphoid cells with a nuclear size equal or exceeding the normal macrophage nuclei. However, even by simple histological examination, considerable heterogeneity can be seen and several morphological variants are described. (3)

Immunophenotypic, tissue microarray and molecular studies underline the
extreme heterogeneity of DLBCLs and suggest a sub classification of the tumor, based on the identification of different pathogenic pathways, which might have much greater relevance than pure morphology for precise prognostic previsions and adoption of ad hoc therapies. The more recent acquisitions on the pathobiology of DLBCLs are reviewed in the light of the authors’ experience, aiming to contribute to the existing debate on the topic. (4,5)

2. Methods of study
The authors should provide the immnohistochemical method of the study.
New revised version:
Immunhistochemical staining technique used in our case was Avidin-Biotin Complex (ABC), as a standard IHC method.

3. DISCUSSION
I think the discussion it is short.
The results should be compared with there of other authors.
New revised version:
Discussion
All large B-cell lymphomas have been lumped into two categories in the REAL classification published in 1994; diffuse large B cell lymphoma (DLBCL) and primary mediastinal B-cell lymphoma (PMBCL).
In the WHO classification of 2001 and even more in the new WHO classification of 2008 the most convincing variants of DLBCL have therefore been separated based on the belief that these variants represent distinct clinico-pathologic entities. (14, 15)

Our case had to be differentiated from other variants of DLBCL such as T-cell / histiocyte rich large B cell lymphoma that shows CD20+, CD30-, CD15- and almost no small CD20+, IgD+ B cells, often more CD8+ than CD4+ T cells in background, as well as from PMBCL.
Employing various immunohistochemical antibodies, such as CD10, CD138, anti-Bcl-2, anti-Bcl-6, MUM1 and anti-p53, several groups have aimed at sub classifying DLBCL into the GCB and ABC subgroups with comparable differences in clinical behavior. (16)
Alizadeh AA et all. has identified two molecularly distinct forms of DLBCL which had gene expression patterns indicative of different stages of B-cell differentiation.
One type expressed genes characteristic of germinal centre B cells ('germinal centre B-like DLBCL'-GCB); the second type expressed genes normally induced during in vitro activation of peripheral blood B cells ('activated B-like DLBCL'-ABC). Patients with germinal centre B-like DLBCL had a significantly better overall survival than those with activated B-like DLBCL. (17, 18)
The patients with GCB subtype of DLBCL had better prognosis than the
non-GCB subtype. Both ABC and GCB DLBCL show a significant improvement of overall survival after R-CHOP treatment. (19, 20)

Our case of ABC DLBCL, underwent R-CHOP treatment and is alive.

Overexpression of bcl-6 protein caused by bcl-6 gene rearrangement may play some important roles in the development and/or progression of a subset of DLBCL. (21)

The group with pattern B (ABC) demonstrated more frequent expression of Ki-67, cyclin D3, geminin, and showed higher proliferative activity than the group with pattern A. These findings suggest that high proliferative activity of tumors with pattern B may be associated with aggressive tumor behavior and poor clinical outcome in patients with DLBCL. (22)

Commonly observed genetic abnormalities that likely contribute to pathogenesis include translocations of BCL6, BCL2, cMYC, and FAS(CD95) mutations, and aberrant somatic hypermutation. Additional novel therapies under investigation include those targeting BCL6 and BCL2, as well as development of novel monoclonal antibody-based therapies (1)

Primary mediastinal large B cell lymphoma (PMBCL) has been thought of as a special subtype of DLBCL. Its distinct clinical presentation in younger patients with a female predominance has led to the suspicion that it constitutes a unique entity. However, reliable distinction from DLBCL has remained elusive. (23)

Grade 3 follicular lymphoma (FL3) has been subdivided into grades 3a and 3b and the percentage of involvement by diffuse large B-cell lymphoma (DLBCL) should also be reported. The clinical implications of these features are unclear. However, patients with FL3 having a diffuse component of more than 50% have an inferior survival that is similar to the survival of those with DLBCL. (24)

While in children Burkitt lymphoma (BL) and DLBCL types probably do not differ clinically, and the differential diagnosis between BL and DLBCL may theoretically appear clear-cut, in adults daily practice shows the existence of cases that have morphological features, immunophenotypic and cytogenetic intermediate between DLBCL and BL, and cannot be classified with certainty in these categories. (25)

There has been discussed the overlap between Burkitt lymphoma and DLBCL; mediastinal gray zone lymphoma and other lymphomas with atypical immunophenotype, gray zone around NLPHL, TCHRLBCL and CHL, EBV positive lymphomas, lymphomas occurring in HIV+ individuals, and PTLD-related B-cell lymphoproliferations, DLBCL with unusual immunophenotype. It became clear that the “double-hit” cases (a combination of a MYC breakpoint with mostly BCL2 breakpoints and other recurrent chromosomal breakpoints), often with distinct morphological features of BL should fall in a novel category of “B cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL. (26)

The main issue addressed during the workshop was to define criteria to reliably distinguish these entities such as NLPHL and THRLBCL, and the gray zones between CHL and DLBCL, mainly THRLBCL in lymph nodes. (26)
“Gray-zone” lymphoma that has been used to denote a group of lymphomas with overlapping histological, biological, and clinical features between various types of lymphomas remains the main diagnostic problem for pathologists.

Reviewer: Antonino Carbone

Major criticisms
1. The Introduction section should be restricted to the morphological aspects and immunohistoprognostical data. Furthermore, it should be more specifically addressed to the differential diagnoses related to clear cell neoplasms.
2. In addition the Introduction section should be reduced by at least one third.
3. The report of the case lacks of radiological findings. In particular, data on the clinical status of the mediastinum are not reported.

Minor criticisms
1. Figures are redundant. This reviewer suggests to maintain only the Fig.s 1, 2, 3, 4, 7. However, these figures should be technically improved.
2. There are a lot of typographical errors.
3. References should be completed.

New revised version:
1, 2. Introduction (shortened and addressed suggestions)

Diffuse large B-cell lymphoma (DLBCL) displays striking heterogeneity at the clinical, genetic, and molecular levels. (1) Diffuse large B-cell lymphoma (DLBCL) is the commonest type of lymphoid tumor world-wide. This category was included both in the REAL and WHO Classification aiming to lump together all malignant lymphomas characterized by the large size of the neoplastic cells, B-cell derivation, aggressive clinical presentation, and the need for highly effective chemotherapy regimens. (2) These tumors are detected as primary or secondary forms both at the nodal and extra nodal levels, in immunocompetent hosts as well as in patients with different types of immunosuppression. They display a significant variability in terms of cell morphology and clinical findings, which justifies the identification of variants and subtypes. (2) Diffuse large B-cell lymphoma (DLBCL) is a diffuse proliferation of large neoplastic B lymphoid cells with a nuclear size equal or exceeding the normal macrophage nuclei. However, even by simple histological examination, considerable heterogeneity can be seen and several morphological variants are described. (3)

Immunophenotypic, tissue microarray and molecular studies underline the extreme heterogeneity of DLBCLs and suggest a sub classification of the tumor, based on the identification of different pathogenic pathways, which might have much greater relevance than pure morphology for precise prognostic previsions and adoption of ad hoc therapies. The more recent acquisitions on the pathobiology of DLBCLs are reviewed in the light of the authors’ experience,
Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin’s lymphoma. The International Prognostic Index (IPI) is useful in predicting the outcome of DLBCL patients. The discovery of specific genetic alterations and the assessment of protein expression led to the identification of multiple novel single molecular markers capable of predicting the outcome of DLBCL patients independently of clinical variables. (6)

However, much confusion exists in the literature regarding the importance of different prognostic biomarkers and their applicability in routine practice. (7, 8, 9, 10, 11, 12, 13)

3. Case presentation

39 years old, white man, Albanian from Kosovo presented with rapidly enlarged lymph nodes in the neck, but also disclosed B symptoms and fatigue. Peripheral blood examination revealed no changes. A cytological aspirate of the lymph node disclosed pleomorphic features. The patient underwent a cervical lymph node biopsy (large excision).

This case was suspected for cervical lymph node metastatic carcinoma, according to first biopsy result on H&E stained slides by other pathologist. Therefore has been undertaken the investigation for the primary carcinoma, which has not been identified. Clinically and radiographically mediastinum was clear. This case has been reviewed by other pathologists (authors), who concluded the final diagnosis as a diffuse large B cell lymphoma-clear cell variant.

4. Figure legends (reduced figures)

Fig. 1. Marked sclerosis and hyalinization in diffuse large B-cell lymphoma. (H&E, 20x)

Fig. 2. Sheets of large cells with abundant pale cytoplasm separated by collagenous fibrosis. Nuclei are round (centroblast-like) or sometimes multilobated. (H&E, 40x)

Fig. 3. CD20+ expressed strong positivity

Fig. 4. bcl-2+, expressed cytoplasmic staining

Fig. 7. Ki67 +, expressed high proliferative index (80%)

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