Challenge of Interpreting Cytoplasmic CD20 Stain in Diffuse Large B Cell Lymphoma Biopsy Specimen of Patients with Steroid Usage

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August 8, 2021

Abstract

Lymphoma work-up involves immunohistochemical stains to help reach the diagnosis. It is imperative to have clinical information and sound knowledge of staining pattern of antibodies to avoid misinterpretation of results. We describe two cases in which pre-biopsy steroid hindered antigenic profile, leading to "cytoplasmic granular staining" and causing delay.

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All authors declare no conflict of interest.

Consent: Please consider waiver No patient details are being shared.

There was no source of funding involved.

Key words: Cytoplasmic, CD3, CD20, Steroid, biopsy, lymphoma, oncology

Author Contributions:

Yang Shi and Yanhua Wang worked-up the cases and reviewed the manuscript. Jui Choudhuri collected material and drafted the manuscript.

Key clinical message:

Pre-biopsy steroid to lymphoma patients alters morphology and can change antigenic profile of neoplastic cells. This hinders interpretation of immunohistochemical stains leading to misdiagnosis. Clinical communication is imperative to avoid this.

Abstract:

Lymphoma work-up involves immunohistochemical stains to help reach the diagnosis. It is imperative to have clinical information and sound knowledge of staining pattern of antibodies to avoid misinterpretation of results. We describe two cases in which pre-biopsy steroid hindered antigenic profile, leading to "cytoplasmic granular staining" and causing delay.

Manuscript:

Introduction : Diffuse Large B-Cell Lymphoma (DLBCL) is the most common type of Non-Hodgkin lymphoma. Patients may have a variable clinical presentation and it may be nodal or extranodal. The 4^{th} edition World Health Organization (WHO) guidelines for tumors of hematopoietic and lymphoid tissues, 2008 defined DLBCL as neoplasm of large B-cell arranged in a diffuse pattern.[1] In 2016 it was revised and updated to include details on cell of origin classification (COO), CD5 expression on prognosis, double expressor lymphoma (DEL) and further understanding of high grade-B cell lymphoma.[2]

The diagnoses for most cases of lymphoma are managed with biopsies and the support of immunohistochemical (IHC) markers. CD3 and CD20, are membranous stains and the most basic initial step in identifying T and B cell lymphoma. However, the diagnosis can sometimes be challenging, like the cases described here where steroids may alter the antigen expression of the neoplasm and present a major challenge clinically and diagnostically for pathologists. We have discussed the implications of steroid use and the importance of sound knowledge of staining patters of different antibodies to correctly interpret membrane and nuclear staining antibodies also highlighting the importance of proper controls.

Case Presentation

First Case: A twenty-five-year-old male presented to the emergency department with lower back pain for three months. It further escalated leading to progressive weakness of the lower limbs. Magnetic resonance imaging (MRI) revealed a large dorsal epidural lesion measuring 6cm in greatest dimension at the level of L4-5 displacing the thecal sac**.** There was severe compression of the thecal sac due to the lesion. According

to radiology the differential diagnoses included hematoma, lymphoma, infected arachnoid cyst, primary neuro-ectodermal tumor (PNET) and metastasis. Neurosurgery was consulted and the patient was started on immediate steroid therapy, with dexamethasone 10mg every six hours to provide immediate relief for the pressure symptoms and meanwhile the patient was worked up for earliest surgical resection of the lesion. The patient received three doses of dexamethasone prior to his surgery.

The surgical specimen revealed diffuse proliferation of lymphoid tissue with fibrosis and necrosis, on hematoxylin and eosin staining. The lesion consisted mostly of small lymphocytes with scattered larger cells and there were numerable apoptotic bodies noted. **(Figure 1)** The initial IHC panel showed unusual cytoplasmic granular staining for CD3, CD19 and CD20, while the controls were unremarkable, making it difficult to interpret the stains. A more extensive panel, showed CD10, CD21, CD30, MUM1, MYC, Cyclin D1, LMP1, CD34, CD56, synaptophysin, chromogranin, ALK1, AE1:3 and TdT to be negative. The large cells occasionally stained for BCL6, PAX5, P53 and CD79a. BCL2 was positive in the smaller lymphocytes and very occasionally in the larger cells. Histologically the lesion was suspicious of a large B-cell lymphoma. Fluorescence in-situ hybridization (FISH) on formalin-fixed, paraffin embedded (FFPE) tissue sections revealed polysomy (3-5 copies) of BCL6 (3q27), MYC (8q24), and BCL2 (18q21.3) in 44-55% interphase nuclei. There was no rearrangement of BCL6, MYC, and BCL2 probes. It was diagnosed as a lymphoma with overexpression of the MYC, BCL2 and BLC6 proteins which is termed as Double Expressor diffuse large B cell lymphoma . Modified R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) and intrathecal therapy was initiated for the patient.

Second case: A fifty-four-year-old woman presented with a neck mass which had been progressively increasing in size in the past one month. Computed tomography (CT) scan revealed multiple enlarged lymph nodes in the neck. The most prominent was a 2.3cm lymph node between the right jugular vein and carotid artery (Level 3). Patient also had enlarged lingual tonsils and underwent a laryngoscopic biopsy for the same and excision of the Level 3 lymph nodes. Patient was administered 4mg dexamethasone intraoperatively to avoid edema during the procedure. This information was not conveyed to the pathologists.

The surgical pathology specimen revealed diffuse proliferation of medium to large pleomorphic lymphoid cells that had completely effaced the nodal architecture. The atypical lymphoid cells had round to oval nuclei with vesicular nuclear chromatin, distinct nucleoli, and moderate amount of cytoplasm. Mitoses and apoptotic bodies were frequently seen and there were extensive areas of necrosis. CD3 and CD20 were challenging to interpret due to the similar staining pattern as described in the previous case. **(Figure 2)** The IHC panel helped diagnose it as DLBCL, with positive staining for PAX5, BCL2, BCL6 and rarely p53 and Ki67 of 60%. The CD10, CD30, MUM1 and MYC were negative in the neoplastic cells. FISH revealed polysomy of MYC, BCL2 and BLC6 proteins in 50-60% of interphase nuclei with no rearrangement.

Discussion : DLBCL is the most common and aggressive Non-Hodgkin B-cell lymphoma, accounting for 30-40% of B-cell lymphoma in the United States.[3] It is a heterogeneous entity with various forms of clinical presentations and response to treatment. The lymphoma cells in DLBCL are characteristically large and diffuse in growth pattern, effacing the normal nodal or extranodal architecture. DLBCL like all lymphoma work ups starts with B and T cell antibodies (for example CD3/CD20). The neoplastic cells show positivity for the pan-B cell markers, CD19, CD20, CD22, PAX5 and OCT2.Pre-diagnosis treatment such as radiation or steroid is a clinical dilemma.[4]It might be essential and lifesaving in some situations especially when there is respiratory compromise due to a lesion or as in the first case threat of neurological damage.[5,6] However the treatment can have major effect on the antigen expression of lymphomas and lead to a challenge in diagnosis. The rationale for steroid administration before biopsy is to reduce tumor burden, thereby minimizing morbidity and this has particularly discussed in patients requiring intubation.

Steroid treatment can lead to complete disappearance of the lesion or may show increased apoptotic bodies and necrosis. Giannini et al. in their review of primary central nervous system lymphoma (PCNSL) discussed the various ways in which steroids can impact tumor morphology ranging from appearance of frequent apoptotic bodies to complete disappearance which is termed as “vanishing” lymphoma.[7] They have described the presence of granular CD20 stain in patients who had been treated with steroids. Interestingly we saw a

similar pattern of expression in the initial IHC stains for CD3, CD19 and CD20. However, without knowing the clinical management, the staining pattern of CD markers could be misleading or even lead to a wrong diagnosis. Hence, a thorough clinical history and seeking information of steroid use is needed in addition to judicious interpretation of the immunostain. This is strongly encouraged when a cytoplasmic staining pattern is encountered. Staining for the other nuclear markers such as PAX5 was interpretable in our cases.

Borenstein et al. have studied the effect of pre-biopsy steroid therapy in pediatric patients with mediastinal lymphoma. They reported an adverse effect on the pathological diagnosis in 22% of their patients.[8] They suggest that preoperative steroids lead to distortion of cellular morphology, compromising accuracy of diagnosis and more importantly delaying or altering diagnosis and management plans. In their study the diagnostic accuracy was not affected for any patients and it was interesting to note that they did not find any significant difference based on the dose and duration of steroid used.[8] Our 1st patient had received a higher dose of steroid and for a longer duration as compared to the 2nd patient. The morphological and IHC impact was more evident on the 1st case, however it is noteworthy that an intra-operative administration of steroid also led to a similar pattern. Two cases are not enough to completely understand the dose and duration which can impact the cell morphology and antigen profile, but it may be sooner than expected. The administration of steroids increased the number of stains required to reach the diagnosis and led to delays.

Conclusion: These cases highlighted the impact of pre-biopsy steroid therapy on pathological diagnostic accuracy. As discussed in previous literature the final diagnosis may not always be affected but it impacts the patient adversely through delay in reaching the final diagnosis. Also, for training pathologists it is important to study controls and know the history before interpreting such cases to avoid errors which can impact decisions on immunotherapy requiring accurate information about antigen positivity. However, the administration of steroid is a clinical necessity in some cases and for these it is important for pathologists to be aware of these changes which might be encountered.

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Figure 1 : Patient 1. Diffuse proliferation of small lymphocytes with occasional larger cells (a, Hematoxylin and eosin stain, 100X) with high nucleus/cytoplasmic ratio and frequent apoptosis (b, Hematoxylin and

eosin stain, 400X). Cytoplasmic staining CD19 (c, 200X), PAX5 (d, 200X), CD79a (e, 200X) and Ki67 (f, 200X).

Figure 2 : Patient 2, Diffuse lymphocytic proliferation (a, Hematoxylin and eosin stain, 100X and b, Hematoxylin and eosin stain, 200X), CD3 cytoplasmic staining (c, 200X), CD20 cytoplasmic staining (d, 200X).