

March 18, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS manuscript-8949- edited).

Title: Manifestations of Gastrointestinal Plasmablastic Lymphoma - A Case Series with Literature Review

Author: Lynette Luria, Johnny Nguyen, Jun Zhou, Michael Jaglal Jane L. Messina, Domenico Coppola, Lubomir Sokol, Ling Zhang,

Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers as shown adequate answers before and edited manuscript in separate file.

Reviewer 1:

This article is written very well as pathological case report. But I had some comment as digestive physician. Case1 In this case, you should show the endoscopic image and CT image. Case2 In this case, you should show the endoscopic image. Case3 Is this case a small intestinal Crohn's disease? Please indicate the positional relationship between the tumor and Inflammatory lesions of Crohn's disease. You should show the CT image. How many years did this patient take a medicine of 6MP?

Answers:

Thanks for the comment. We have gone back to retrieve and review all available clinical information at our institute.

- 1) Endoscopic images for Cases 1 and 2 before surgical resection are not available as these patients were initially evaluated and had initial interventions at outside institutions.
- 2) Case 3 - Crohn disease was diagnosed in 1999 and primarily involves the terminal ileum. The patient was initially managed with Asacol, Imuran, Pentasa and prednisone until 2001. He underwent an ileostomy was performed with 18 inches of small bowel removed. Pathologic examination shows no malignancy. He has been on corticosteroid (Entocort) and 6-mercaptopurine (6-MP) for Crohn's flares over 10 years. Because of fistula-ex-ano, a cyto suture was placed. The previous imaging study showed characteristic features of Crohn disease. A CT study of abdomen in 2011 before surgery revealed diffuse bowel wall thickening of small bowel loop and associated with a mass showing irregular central area of

non-enhancement measuring 7.0 x 4.0 cm around the small bowel. A segment of small bowel (31 cm in length, and 5.2 to 9.1 cm in circumference) proximal to the prior ileocolostomy site was removed. Gross examination showed discoloration in the segment of small bowel measuring 9.5 cm located 6.0 cm from the un-oriented surgical margin. Microscopic examination showed segmental involvement by PBL through the whole thickness of bowel wall, which was superimposed with inflammatory process. The uninvolved segments showed typical features of Crohn disease.

Reviewer 2:

This case report is well written. Minor revision Discussion “One of the reasons patients with plasmablastic lymphoma may not present B symptoms can be explained by a proposed mechanism for its pathogenesis. “ Please clarify the reasons.

Answer:

In the discussion, we have provided clarification as to why patients with PBL may not present with B symptoms.

Reviewer 3:

The authors have presented three cases of primary GI large B-cell lymphomas with immunophenotype and EBV expression consistent with plasmablastic lymphoma. Only one of the three cases were HIV positive and a second patient had iatrogenic immunosuppression. They have also summarized clinicopathological findings of 14 cases of primary GI plasmablastic lymphoma review of literature. They discuss the possible molecular and immune mechanisms that may lead to this uncommon tumor. Since the entity of plasmablastic lymphoma was first described and has been best characterized in HIV+ patients, case #1 is prototypic. Case 2- Confirm whether the lymphoma was positive for CD38 or CD138 and whether CD79a was negative or not tested (different information in text and in Table 1). If the tumor was CD20 negative, why was Rituxan used in the treatment? Any history of immunosuppressive therapy in this patient? Why this is not EBV related DLBCL of elderly? General Comment: The discussion should include the differential diagnostic considerations and the immunophenotype and clinical features which may be useful to distinguish them from plasmablastic lymphoma. A uniform battery of immunostains, including EBV LMP, EBNA2, HHV8, MUM1, and other pan-B antigens, should be performed and reported on the three cases. The cell morphology from all cases should be compared side by side. This basic documentation will increase the value of this small case series.

Answers:

- 1) We updated the text and table to reflect the fact that in Case 2, CD138 and CD79a were both negative. Bright CD38 and VS38 expressions were reported.
- 2) We updated the text to explain that the patient in Case 2 was treated with Rituxan because the IHC panel received from the referring entity did not contain a CD20 stain. The clinician thought it would be prudent to add Rituxan to the patient's therapy until our institution evaluated the slides and ran additional IHC.
- 3) Case 2. There is no history of immunosuppressive therapy for the patient before he

developed PBL.

- 4) In the summary of Case 2, we describe the immunophenotype of lymphoma cells that make a diagnosis of EBV-positive diffuse large B cell of the elderly less likely as a diagnosis and added a reference to Ok et al. which describes the IHC markers for EBV+ DLBCL, often CD10 (-), BCL-6 (-) and MUM1(+) (non-germinal center cells type).
- 5) We have included additional background information on Plasmablastic Lymphoma to the Discussion section of our paper including: differential diagnosis, immunophenotypes, and clinical features of the disease.
- 6) It is not possible for us to perform a uniform battery of immunostains on all 4 cases because the original tissue samples and slides sent to us for evaluation have been returned to each of the referring institutions.
- 7) All the case studies from our institution contain a brief morphological description of each patient's tumor.

Reviewer 4:

<<Abstract>>

#1 delete (table 1) and (14cases) in abstract.

<<Introduction>>

<1st paragraph>

#2 change "plasmablastic lymphoma" to "plasmablastic lymphoma (PBL)" and replace to "PBL" in

following text.

#3 change "EBV" to "Epstein-Barr virus (EBV)"

#4 change "MYC gene" to "MYC (*Italics*) gene"

<Case 1>

#5 change "Grams/L" to "g/L" or "g/dL"

#6 change "Bcl-2/bcl6" to "BCL2/BCL6"

#7 change "ALK-1" to "ALK"

#8 change "HHV8" to "human herpesvirus 8 (HHV8)"

#9 change "PET" to "fluorodeoxyglucose positron emission tomography (FDG-PET)"

#10 change "Septra" to "trimethoprim-sulfamethoxazole"

<<Case 2>>

#11 change "Grams/L" to "g/L" or "g/dL"

#12 change "chemistry prolife" to "chemistry profile"

#13 change "Mu" to "IgM"

#14 change "BCL-2/bcl-6/CDX-2" to "BCL2/BCL6/CDX2"

#15 change "positron emission tomography (PET)" to "FDG-PET"

#16 change "autologous hematopoietic stem cell transplant" to "autologous hematopoietic stem cell

transplant (auto-HSCT)"

Answers:

All grammatical and formatting changes suggested by this reviewer were incorporated into our text.

Reviewer 5:

. Dear Authors I read with interest the article of Nguyen et al. entitled "Manifestations of Gastrointestinal Plasmablastic Lymphoma - A Case Series with Literature Review". More than 14 cases are described in the literature. Plasmablastic lymphoma (PBL) could be confounded to anaplastic plasmacytoma. As stressed in this article EBER reactivity distinguishes PBL from the other lymphomas. Lack of pan-B cell expression differentiates PBL from DLBCL with immunoblastic or plasmacytic features. MYC expression could have an important impact on future targeting therapies. But I have some questions about this article. In the discussion there are some sentences that describe symptoms and signs of the three patients reported in the study and of patients reported from literature. For example "B symptoms are symptoms associated with B-cell lymphoma and include weight loss, fever.....Our cases and literature review of GI plasmablastic lymphoma showed weight loss in 50% of the patients on presentation, fever in 22%, and night sweats in 6%. Overall, 67% of the patients displayed B or "Only one patient displayed all three B symptoms, and this patient was HIV-positive. All patients with GI plasmablastic lymphoma had..... All these informations must be removed and added in the subchapter "Literature Review of Gastrointestinal Plasmablastic Lymphoma". These data report clinical symptoms of all the population studied. In the article of Castillo et al. was described a large series of PBL HIV positive patients with oral PBL and rearrangements of v-myc oncogene (MYC) were disclosed in 41% of the tested patients. In the article of Nguyen only one HIV patients disclosed MYC (no performed in other cases reported). Furthermore, in the article of Bogusz et al. it was demonstrated that MYC expression was more frequent in HIV infected patients with a number of CD4+ cell count less than 300/mm³. Higher frequency of MYC expression in HIV infected patients and in particular in patients with lower CD4+ count must be commented. The article of Bogusz et al. published on Am J Clin Pathol. 2009 Oct;132(4):597-605 describe a series of HIV infected patients with PBL, one of this patient had ano-rectal PBL and must be included and described in the present article. I believe that this article could be of interest for the reader.

Answers:

- 1) The percentages of B symptoms identified in our literature review of GI PBL case studies was moved from the Discussion section and added to the Literature Review of Gastrointestinal Plasmablastic Lymphoma section. It was initially located in the Discussion section because it also incorporated our case studies into the percentages.
- 2) It is true that the Valera et al. study compiled a much larger pool of PBL cases to investigate c-MYC as a potential contributor to PBL's aggressive nature and that in our review, only one patient was evaluated for the c-MYC rearrangement. We thought it important to cover c-MYC in the discussion as it is still being evaluated and it may be beneficial to future research efforts for pathologists and clinicians to test their future PBL patients for this rearrangement.
- 3) The additional information from Bogusz et al. requested by the reviewer was added to the discussion section.

Reviewer 6:

The authors report 3 patients with plasmablastic lymphoma, presenting under 3 different scenarios. Recommend using updated reference for mechanisms of EBV infection: OK, Blood 2013; 122: 328. Minor issues: 1. Case 1 in abstract and report: it is redundant to state extranodal and rectal mass 2. Introduction: Remove "lymph nodes" from extranodal sites at the end of first paragraph. 3. Results case 1: HAART is not used any longer: ART is currently used. 4. Case 2: Change Hemoglobin to 150 g instead of 15 g 5. Case 3. State length of survival after diagnosis. 6. Add your data to Table 2 and Table 3, and when summarizing data, refer to 17 cases instead of 14 cases. 7. Revise magnification of figures 1E and 1F. Cells appear clearly smaller than cells in figures 1C and 1D which are noted to be x600 magnification. 8. Table 2. There are 2 NR= none reported and NR= Non responder (therapy); needs to modify one of the NR.

Answers

We have fixed the errors.

- 1) The reference "EBV-positive diffuse large B-cell lymphoma of the elderly" by Ok et al. was evaluated per the reviewer's request and information regarding the role of LMP1 in EBV's oncogenic abilities was added to the Discussion section.
- 2) The redundancies and grammatical changes suggested by this reviewer were incorporated into our paper.
- 3) Length of survival - All available survival data for our 4 cases are included in Table 1.
- 4) Our data from Table 1 was incorporated into Tables 2 and 3 (now called Tables 1 and 2 respectively).
- 5) The captions for our figures were updated to reflect the correct magnifications.

Also, please note that we have added Dr. Michael Jaglal, MD, to the authorship list and have cited his contribution in the accompanying revised manuscript. Also, because Dr. Messina is currently out of town at the time of resubmission, we have not been able to enclose the Copyright Assignment, which we will send to you as soon as possible upon her return.

Again, thank you for consideration of our manuscript for the *World Journal of Gastroenterology*.

Sincerely,

Ling Zhang, MD

Department of Hematopathology and Laboratory Medicine

H Lee Moffitt Cancer Center and Research Institute

Tampa, FL 33612, United States.

Ling.zhang@moffitt.org

Johnny Nguyen, MD

Department of Pathology, University of South Florida Morsani College of Medicine, MDC

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Tampa, FL 33612, United States.

jnguyen@health.usf.edu

Telephone: +813-396-2515

Fax: +813-905-9896