

July 10, 2015

Dr. Ya-Yuan Ma  
Science Editor, Editorial Office  
Baishideng Publishing Group Inc.

RE: World Journal of Gastroenterology Manuscript # 20319

Dear Dr. Ya-Yuan Ma:

Thank you for your careful review and thoughtful criticisms of the manuscript entitled, "Proposed novel syndromic pentad for Aspergillus appendicitis: Case report and literature review" by Mihajlo Gjeorglievski, Mitual Amin, and Mitchell S. Cappell submitted as manuscript #20319 to World Journal of Gastroenterology. This work has been entirely revised in accord with the reviewers' criticisms as follows.

1. Reviewer # 00731737

Criticism 1: *Very nice case report - Histopathological findings and figures are well presented - Discussion is well conducted - Could authors discuss the role of Filgrastim and its impact on clinical response*

Response to criticism:

Page 7, Top.

As suggested the following IS ADDED:

This work illustrates that neutropenia after chemotherapy is an important risk factor for Aspergillus appendicitis/enterocolitis, and emphasizes the importance of filgrastim therapy to decrease the severity or duration of neutropenia after chemotherapy. Filgrastim helps prevent febrile neutropenia from Aspergillus or other opportunistic infections in experiments in mice [32,33] or in clinical trials [34,35], and is likely important in treating Aspergillus appendicitis or enterocolitis occurring in the setting of neutropenia.

ADDED REFERENCES:

32. **Arber C**, Bitmansour A, Shashidhar S, Wang S, Tseng B, Brown JM. Protection against lethal Aspergillus fumigatus infection in mice by allogeneic myeloid progenitors is not major histocompatibility complex restricted. *J Infect Dis* 2005; **192**: 1666-71. PMID: 16206084.
33. **BitMansour A**, Cao TM, Chao S, Shashidhar S, Brown JM. Single infusion of myeloid progenitors reduces death from Aspergillus fumigatus following chemotherapy-induced neutropenia. *Blood* 2005; **105**: 3535-7. PMID: 15576478.
34. **Aapro MS**, Bohlius J, Cameron DA, Dal Lago L, Donnelly JP, Kearney N, Lyman GH, Pettengell R, Tjan-Heijnen VC, Walewski J, Weber DC, Zielinski C; European Organisation for Research and Treatment of Cancer. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. *Eur J Cancer* 2011; **47**: 8-32. doi: 10.1016/j.ejca.2010.10.013. PMID: 21095116
35. **Weycker D**, Barron R, Edelsberg J, Kartashov A, Legg J, Glass AG. Risk and consequences of chemotherapy-induced neutropenic complications in patients receiving daily

filgrastim: the importance of duration of prophylaxis. *BMC Health Serv Res* 2014; **14**: 189. doi: 10.1186/1472-6963-14-189. PMID: 24767095

2. Reviewer # 03364660

Criticism 1. *"Aspergillus appendicitis" is enough to be reported but its "syndromic pentad" is not the improper term. Four of 5 syndromic pentad is not unique characteristic of Aspergillus appendicitis but common feature of neutropenic appendicitis or enterocolitis (typhilitis): (1) clinically-suspected appendicitis, (2) neutropenia, (3) recent chemotherapy, (4) underlying acute leukemia I think that this case report focused on two unique clinical features of "isolated aspergillus appendicitis" differentiating from other neutropenic appendicitis or enterocolitis The first feature is "poor response if administered antibacterial antibiotics or anti-Candidal therapy without anti-Aspergillus therapy". The other is relatively better prognosis than other Aspergillus appendicitis combined with enterocolitis. Therefore, I recommend the authors to correct the title and conclusion according to above my comments.*

A. Page 1.

As suggested,

TITLE CHANGED TO:

Characteristic clinical features of *Aspergillus appendicitis*: Case report and literature review

CHANGED FROM:

Proposed novel syndromic pentad for *Aspergillus appendicitis*: Case report and literature review

B. Page 2, Core Tip, line 2.

CHANGED TO:

and identifies a clinical pentad associated with this syndrome...

CHANGED FROM:

and identifies a novel pentad associated with this syndrome...

C. Page 2, Core Tip, line 7.

CHANGED TO:

presenting with this proposed pentad, *Aspergillus appendicitis* should be considered in the differential diagnosis,

CHANGED FROM:

presenting with this novel proposed pentad, *Aspergillus appendicitis* should be considered,

D. Page 3, Bottom. Abstract, Conclusions.

As suggested,

CHANGE TO:

Based on these four cases, a clinical pentad is proposed for *Aspergillus appendicitis*

CHANGED FROM:

Based on these four cases, a novel syndromic pentad is proposed for *Aspergillus appendicitis*

E. Page 6, Top.

As suggested,

CHANGE TO:

To promote early diagnosis and appropriate treatment, a clinical pentad is proposed for suspected *Aspergillus* appendicitis of:

CHANGED FROM:

To promote early diagnosis and appropriate treatment, a syndromic pentad is proposed for suspected *Aspergillus* appendicitis of:

F. Page 6, Bottom.

As suggested,

CHANGE TO:

The importance of early anti-*Aspergillus* therapy is emphasized by two of the four reported cases of isolated *Aspergillus* appendicitis improving dramatically after instituting anti-*Aspergillus* therapy and appendectomy. Of note, the prognosis of isolated *Aspergillus* appendicitis appears to be better than that of *Aspergillus* appendicitis combined with *Aspergillus* enterocolitis.

CHANGED FROM:

The importance of early anti-*Aspergillus* therapy is emphasized by two of the four reported cases improving dramatically after instituting anti-*Aspergillus* therapy and appendectomy.

G. Page 7, Bottom.

As suggested,

CHANGE TO:

All five criteria appear to be typical for *Aspergillus* appendicitis, but the first four criteria may also occur with (bacterial) neutropenic typhlitis/enterocolitis and only the fifth criterion of poor response to conventional therapy is relatively specific for *Aspergillus* appendicitis.

CHANGED FROM:

Addition of the fifth criterion of poor response to conventional antibiotic therapy is designed to increase the suspicion of *Aspergillus* infection instead of bacterial neutropenic typhlitis/appendicitis.

*Criticism 2. CT images on Figures 1A and B is not enough to demonstrate this case. I want to replace more qualified CT scans of axial scan with less than 5mm slice thickness for adequate demonstrate periappendiceal inflammation (periappendiceal fat stranding and infiltration adjacent right psoas muscle).*

CHANGED TO:

Added two new CT images as follows

Figure 1C, which demonstrates periappendiceal inflammation (periappendiceal fat stranding).

Figure 1D, which demonstrates enlarged right psoas muscle with indistinct margins due to inflammatory infiltration.

CHANGED FIGURE LEGENDS OF FIGURE 1 TO:

Figure 1A-D. CT images of abdomen using IV non-ionic contrast and standard oral contrast demonstrates dilated appendiceal ostium and thickened appendiceal wall in cross-section (arrows in A); dilated lumen and thickened wall of vermiform appendix in longitudinal section (arrows in B); periappendiceal fat stranding (arrows in C); and an enlarged right psoas muscle with indistinct margins from local extension of the appendiceal inflammation (enlarged right psoas muscle with indistinct margins identified in D by 2 arrows, as compared to normal-sized left psoas muscle with distinct margins identified by 1 arrow). The appendix measures approximately 11 mm in diameter from outer wall to outer wall. All these findings are consistent with acute appendicitis. There is no evident appendicolith or typhlitis.

#### CHANGED FIGURE LEGENDS OF FIGURE 1 FROM:

Figure 1A & B. CT images of abdomen using IV non-ionic contrast and standard oral contrast demonstrates dilated appendiceal ostium and thickened appendiceal wall in cross-section (arrows in A), and demonstrates dilated lumen and thickened wall of vermiform appendix in longitudinal section (arrows in B). The appendix measures approximately 11 mm in diameter from outer wall to outer wall. Note presence of periappendiceal fat stranding enlargement and inflammatory changes of the adjacent psoas muscle. All these findings are consistent with acute appendicitis. There is no evident appendicolith or typhlitis.

Criticism 3. *Correction of Figure legends Figure 1A & B. : "fat stranding enlargement" is corrected to "fat stranding".*

#### CHANGED TO:

"fat stranding"

#### CHANGED FROM:

"fat stranding enlargement".

#### CHANGES TO CONFORM TO EDITOR'S CRITICISMS & JOURNAL POLICIES

1. On title page. The manuscript has been revised to provide author's name given first, and then the complete names of institution, city, province, and postcode

2. As per editorial policy the structured abstract is changed to an unstructured abstract.

#### CHANGE TO:

This work aims to facilitate diagnosing Aspergillus appendicitis, which can be missed clinically due to its rarity, by proposing a clinical pentad for Aspergillus appendicitis based on literature review and one new case. The currently reported case of pathologically-proven Aspergillus appendicitis was identified by computerized search of pathology database at William Beaumont Hospital, 1999-2014. Prior cases were identified by computerized literature search. Among 10,980 pathology reports of pathologically-proven appendicitis, one case of Aspergillus appendicitis was identified (rate=0.01%). A young boy with profound neutropenia, recent chemotherapy, and AML presented with RLQ pain, pyrexia, and generalized malaise. Abdominal-CT scan showed a thickened appendiceal wall and periappendiceal inflammation, suggesting appendicitis. Emergent laparotomy showed an inflamed, thickened appendix, which was resected. The patient did poorly postoperatively with low-grade-fevers while receiving antibacterial therapy, but rapidly improved after initiating amphotericin therapy. Microscopic examination of a silver-stain of the appendectomy specimen revealed fungi with characteristic

Aspergillus morphology, findings confirmed by immunohistochemistry. Primary Aspergillus appendicitis is exceptionally rare, with only 3 previously reported cases. All three cases presented with neutropenia, recent chemotherapy, acute leukemia, and suspected appendicitis; the two prior cases initially treated with antibacterial therapy fared poorly before instituting anti-Aspergillus therapy. The current patient satisfied all these five criteria. Based on these four cases, a clinical pentad is proposed for Aspergillus appendicitis: clinically-suspected appendicitis, neutropenia, recent chemotherapy, acute leukemia, and poor clinical response if treated solely by antibacterial/anti-candidial therapy. Patients with this proposed pentad may benefit from testing for Aspergillus infection by silver-stains/immunohistochemistry and considering empirical anti-Aspergillus therapy pending a tissue diagnosis.

#### CHANGED FROM:

**AIM-**Help diagnose Aspergillus appendicitis, which can be missed clinically due to its rarity, by proposing a novel, syndromic pentad for Aspergillus appendicitis based on literature review and one new case.

**METHODS-**Computerized search of pathology database at William Beaumont Hospital, 1999-2014, to identify cases of pathologically-proven Aspergillus appendicitis. Prior cases identified by computerized literature search.

**RESULTS-**Among 10,980 pathology reports of pathologically-proven appendicitis, one case of Aspergillus appendicitis was identified (rate=0.01%). A young boy with profound neutropenia, recent chemotherapy, and AML presented with RLQ pain, pyrexia, and generalized malaise. Abdominal-CT scan showed a thickened appendiceal wall and periappendiceal inflammation, suggesting appendicitis. Emergent laparotomy showed an inflamed, thickened appendix, which was resected. The patient did poorly postoperatively with low-grade-fevers while receiving antibacterial therapy, but rapidly improved after initiating amphotericin therapy. Microscopic examination of a silver-stain of the appendectomy specimen revealed fungi with characteristic Aspergillus morphology, findings confirmed by immunohistochemistry. Primary Aspergillus appendicitis is exceptionally rare, with only 3 previously reported cases. All three cases presented with neutropenia, recent chemotherapy, acute leukemia, and suspected appendicitis; the two prior cases initially treated with antibacterial therapy fared poorly before instituting anti-Aspergillus therapy. The current patient satisfied all these five criteria.

**CONCLUSIONS-**Based on these four cases, a clinical pentad is proposed for Aspergillus appendicitis: clinically-suspected appendicitis, neutropenia, recent chemotherapy, acute leukemia, and poor clinical response if treated solely by antibacterial/anti-candidial therapy. Patients with this proposed pentad may benefit from testing for Aspergillus infection by silver-stains/immunohistochemistry and considering empirical anti-Aspergillus therapy pending a tissue diagnosis.

3. All references have been changed to conform to the format of the World Journal of Gastroenterology. The doi numbers and the PMID numbers have been added for all the references when available.

4. All reference numbers have been placed in square brackets as superscripts.

5. As suggested comments for each subsection have been added on pages 16-17, as follows:  
Comments for each subsection

### **1. Case characteristics:**

An 8-year-old boy with acute myelogenous leukemia (AML), diagnosed one month earlier, and with profound neutropenia, after receiving two cycles of IV chemotherapy with daunorubicin, cytarabine, thioguanine, etoposide and dexamethasone, presented with low-grade pyrexia and chills for one day.

### **2. Clinical diagnosis:**

Physical examination revealed temperature=36.8 °C, pulse=111 beats/min, blood pressure=94/63 mmHg. and mild right lower quadrant (RLQ) tenderness without rebound tenderness. The leukocyte count=200 cells/mm<sup>3</sup> (normal 4,800-10,100 cells/mm<sup>3</sup>), neutrophil count<100 cells/mm<sup>3</sup> (normal 1,600-7,200 cells/mm<sup>3</sup>), hematocrit=36.3% (normal in male boy, age 6-9 years: 33.6-43.4%), and platelet count=90,000/mm<sup>3</sup> (normal 150,000-400,000/mm<sup>3</sup>).

### **3. Differential diagnosis:**

Clinical presentation is an episode of febrile neutropenia. The differential diagnosis includes neutropenic typhlitis and/or appendicitis, due to local opportunistic infections including bacterial infections, Candida, Mucor, Histoplasma, or Aspergillus.

### **4. Laboratory diagnosis:**

The leukocyte count=200 cells/mm<sup>3</sup> (normal 4,800-10,100 cells/mm<sup>3</sup>), and neutrophil count<100 cells/mm<sup>3</sup> (normal 1,600-7,200 cells/mm<sup>3</sup>). These findings demonstrate profound neutropenia.,

### **5. Imaging diagnosis:**

Abdomino-pelvic CT scan revealed a dilated appendiceal lumen, thickened appendiceal wall, and periappendiceal fat stranding, findings consistent with appendicitis.

### **6. Pathological diagnosis:**

Microscopic examination of hematoxylin and eosin stains of histologic sections of the resected appendix showed an acute necrotizing appendicitis, with scattered questionable fungal hyphal forms. A Grocott-Gomori methenamine-silver nitrate stain performed on the resected specimen revealed numerous fungal hyphae that showed features characteristic of Aspergillus of septation and acutely angled branching, and which was confirmed as Aspergillus by immunohistochemistry.

### **7. Treatment:**

The patient underwent appendectomy for the appendicitis and was administered IV filgrastim (granulocyte colony stimulating factor) for the profound neutropenia. On postoperative day 13 IV amphotericin-B therapy was added when Aspergillus appendicitis was identified by special stains performed on the resected appendix.

### **8. Related reports:**

Review of the modern literature revealed only 3 other reported cases of isolated Aspergillus appendicitis, aside from one case very briefly reported in German in 1959.

### **9. Term explanation:**

None required.

### **10. Experiences and lessons:**

Analysis of the current case and review of the three prior case reported in the modern literature, suggest that patients with Aspergillus appendicitis may clinically present with a pentad of: clinically-suspected appendicitis, neutropenia, recent chemotherapy, acute leukemia, and poor clinical response if treated solely by antibacterial/anti-candidal therapy.

### **11. Peer-review:**

The strengths of the article include: reporting a well-documented case of isolated Aspergillus appendicitis, thorough review of the three previously reported cases as identified by review of

the modern literature, and the proposal of a clinical pentad to facilitate clinical recognition of *Aspergillus* appendicitis. The main weakness, as pointed out by peer review, is that only four cases have been reported of isolated *Aspergillus* appendicitis, and therefore the conclusions about the general clinical presentation of this infection are tentative and require further confirmation.

Aside from this point-by-point response letter, please see the final version incorporating all the revisions and the final version showing all the revisions with tracking.

Thank you for your interest in this manuscript. Please note that we are prepared to further revise this manuscript if necessary to satisfy the reviewers' criticisms.

Warm regards,  
Mitchell S. Cappell, M.D., Ph.D.  
Chief, Division of Gastroenterology & Hepatology  
& Professor of Medicine  
William Beaumont Hospital, MOB #602  
3535 West Thirteen Mile Road  
Royal Oak, MI 48073 USA