Peer Review File
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Review Comments

Reviewer A

Comment 1: Highlight box:
'Key findings': The second bullet point in this section seems a repetition of what the first bullet point states. Last section of box not visible in pdf document of manuscript for review ('What is the implication, and what should change now?'- only the first line is visible).
Reply 1: Thank you for taking the time to review our manuscript as well as provide the accompanying feedback and edits. We have removed the second bullet point that seems repetitive. We have made sure in the latest pdf version that the entire highlight box is viewable.
Changes in Text 1: Please see highlight box for the aforementioned changes.

Comment 2: Background: I think a line or 2 to give the reader an overview of leflunomide as a drug- usual indications, mechanism of action etc, would be useful.
Reply 2: This is a good idea, we have included changes that document this in the background section. While this Leflunomide's indications and mechanisms of action was mentioned in the discussion section also, we have edited both the background and discussion sections to avoid sounding repetitive.
Changes in Text 2: Please see background section of abstract and introduction section and discussion sections of the main case report.

Comment 3: Line 48- You have stated that 'leflunomide-induced colitis is a rare entity'. Although colitis is a rare reported side effect of the drug, gastrointestinal side effects are relatively common. Consider adding this plus relevant data, for example 17% reported diarrhea in the leflunomide clinical trials (Ref: Smolen et al. DOI: https://doi.org/10.1016/S0140-6736(98)09403-3).
Reply 3: We appreciate you giving us another reference to contextualize our article, we have incorporated your suggestions in the introduction.
Changes in Text 3: We have rephrased this line as follows: "While the gastrointestinal symptoms of this medication is common and approximately 17% of patients reported diarrhea in clinical trials, leflunomide-induced colitis remains a rare entity."

Comment 4: Line 55-56. Consider re-wording this: 'guide diagnosis all in one setting'.
Reply 4: We have rephrased this sentence to aid in clarity
Changes in Text 4: Changes were made as follows "This case includes a clinical presentation coupled with clear endoscopic and microscopic findings that helped make a diagnosis.

Comment 5: Case description: Please check abbreviation guidance in line with the journal requirements- eg. line 61 COPD, line 71 CBC, BMP, CRP, ESR.
Reply 5: Thank you notifying us of the abbreviations, we have made our best effort to spell out all abbreviations to make reading our manuscript easier.
Changes in Text 5: COPD has been replaced with chronic obstructive pulmonary disease, CBC is now complete blood count, BMP is now basic metabolic panel, CRP is written C-reactive protein and ESR is now erythrocyte sedimentation rate.

Comment 6: Line 74- 'Rocephin, Flagyl'. Provide generic drug name, rather than brand name.
Reply 6: Thank you for letting us know.
Changes in text 6: Rocephin is now ceftriaxone, and flagyl is now written as metronidazole.

Comment 7: Line 63- what dose of leflunomide was she taking?
Was she taking any other medications? This is relevant as there could potentially be other causative drugs contributing to her presentation.
Was there any previous history of gastrointestinal diagnoses or symptoms prior to this current presentation?
Reply 7: She was taking leflunomide 10mg once daily. Prior to hospitalization, her other medications included budesonide and albuterol as needed and denosumab injection every 6 months for osteoporosis. She was not taking NSAIDs.
Changes to text 7: We have included the dosage and frequency of leflunomide this patient was taking in the case description and abstract. We have expanded her past medical history and included the other medications she was taking in the case description section.

Comment 8: Line 73- what was the fecal lactoferrin level?
Reply 8: We did re-check the chart for any available values, unfortunately the lab at our hospital does not report the fecal lactoferrin as a numerical result, it only states if its positive and negative.
Changes in text 8: None

Comment 9:
Figure 1 and 2 of endoscopic findings- please label which part of the colon the photo is referring to.
Reply 9: Thank you under the captions we have included the specific part of the colon and rectum.
Changes in text 9: Please see captions regarding Figures 1 and 2.

Comment 10: Line 84- You have stated that on discharge, her stool frequency had reduced to 2-3 episodes per day, compared to 6 per day on admission. Consider specifying how many days after stopping the leflunomide before symptomatic benefit was seen.
Reply 10: Unfortunately, after taking another look at the patient's hospital documentation, there has not been daily intake and output recording for the patient during the stay that allows us to definitively state when the number of bowel movements decreased to 3-4. We reviewed the physician notes as well and 2-3 episodes is documented in two progress notes on the 3rd to last day of discharge. She was hospitalized for a total of 7 days.
Changes in text 10: None

Comment 11: Has the patient had any further investigations after resolution of symptoms? Eg. further biochemical assessment- CRP/ESR, fecal lactoferrin, or endoscopic assessment?
Reply 11: Patient was referred to follow up with gastroenterology upon discharge, but no follow up labs are available after the hospitalization described in the case report. However, the hospital electronic record does have a colonoscopy from just last month, 4/2023, which was performed to follow-up from last year's colonoscopy as well as patient complaining of rectal pain. Diarrhea was not mentioned. Findings included a diffuse area of mildly congested mucosa with areas of vascular effacement in the sigmoid, descending, transverse and ascending colon. Biopsies were taken from these areas. 1 diminutive mid rectum colon was removed with cold snare. Patient had external and internal hemorrhoids. The biopsies revealed no pathologic abnormalities. The polyp was hyperplastic. As we have not obtained consent to share the latest information we have not included this in the case report at this time.
The most recent hospital records show that sadly the patient died as of earlier this month 5/2023 from being found unconsciousness, brought to the hospital and found to have intracranial hemorrhage. This was unknown to any of the authors until this round of editing commenced. Considering how recent this was, the authors feel it may be more appropriate to leave the case report details as they are now, since obtaining consent from family members at this time would be insensitive to the loss of their family member. However, if the editors feel this may be valuable to the case please let us know and we can attempt to contact the patient's family for consent regarding the latest endoscopy findings.
Changes in text 11: None at this time

Comment 12: Discussion Line 93- 'pathogenesis of colitis yet to be elucidated'. Consider mentioning that leflunomide is a prodrug and turns to its active metabolite form, in the intestinal mucosa and plasma, which may be relevant in the pathogenesis of colitis.
Reply 12: While using some important information on the mechanism of action of leflunomide from the new reference included in the manuscript from Comment 3 (https://doi.org/10.1016/S0140-6736(98)09403-3), we have elaborated on leflunomide's properties as a prodrug and point towards a possible pathophysiological mechanism for colitis.
Changes in text 12: Changes have been made to the sentence originally on line 93 with additional information on leflunomide subsequently as follows "Leflunomide is known as a disease modifying antirheumatic drug (DMARD) which has anti-inflammatory and immunomodulatory properties but the exact pathogenesis of leflunomide colitis has yet to be elucidated. Leflunomide itself is a prodrug and its active metabolite selectively inhibits the enzyme dihydroorotate dehydrogenase, a key enzyme in the synthesis of pyrimidines. Activated T-lymphocytes are one of the cell types that predominantly synthesize pyrimidines and especially sensitive to leflunomide, it is from this effect it is theorized that alterations of the immune system can occur. Perhaps this may play a role in the development of colitis."

Comment 13: Line 94-95- you have provided the frequency of gastrointestinal adverse effects, consider adding the reported frequency of diarrhea specifically as an adverse effect.
Reply 13: Thank you for this suggestion. We think this would be valuable information also. However, after reviewing our references and some additional publications and drug information websites regarding the frequency of diarrhea specifically (i.e 3-6 episodes daily), this information was not available. The data is still presented as percentage of individuals who experience diarrhea, not the frequency of diarrhea once they experience it.
Changes in text 13: None

Comment 14: Lines 101-104. Consider adding that this is similar to the timeline of resolution of symptoms seen in your case- makes the discussion more relevant to the case.
Reply 14: Agreed, clarifying how this was exactly like the timeline in our case will be increase the relevancy of our case report.
Changes in text 14: "The timeline of symptom onset and resolution as mentioned above is very similar in our case." Found in the discussion.

Comment 15: Line 105-6- I think it would be worth mentioning that the colitis changes seen previously in other case reports, eg. Chis et al, were more evident macroscopically, whereas in your case the changes were more consistent with microscopic colitis (increased lymphocytes and thickened collagen subepithelial layer), and there was no mention of crypt abscesses in the histological description of your case.
(Suggested ref re: microscopic colitis in leflunomide: Hamdeh et al. doi: 10.1016/j.cgh.2020.04.069)
Reply 15: This is a helpful additional reference. We have made edits in the discussion that bring up the above findings and differentiating lefunomide induced colitis vs microscopic colitis. We also combine this with our statements on the mechanism for how leflunomide's affects the immune system (combining this with the edits made from comment 12. We truly believe this aided to the depth of our discussion, its relation to the past literature and adding something new to think about regarding the pathophysiology of lefunomide colitis.
Changes in text 15: Found in the discussion section "Furthermore, prior published cases with biopsy findings, mention luminal subepithelial collagenous bands and colonic crypt formation including crypt abscesses. Others may demonstrate biopsy findings that resemble a combination of both lymphocytic and collagenous types of microscopic colitis. The latter is most similar to our case and there was no mention of crypt formation in this patient's biopsy report. There have been instances of microscopic colitis associated with leflunomide as well. The major distinction between microscopic colitis and a drug induced colitis is that microscopic colitis by definition will present with normal mucosa on colonoscopy. The lymphocytic predominance in the biopsy findings may relate back to the possible effects leflunomide has on the activated T lymphocytes, begetting a

pathophysiological mechanism to this condition. There remains a range of endoscopic findings associated with leflunomide colitis, including mucosal hyperemia, ulceration and vasculature effacement similar to this patient's colonoscopy."

Comment 16: Line 112- consider explaining briefly to the reader how a cholestyramine washout may be useful.
Reply 16: We have included additional information the utility of cholestyramine washout at the end of the conclusion section.
Changes in text 16: "For severe colitis with more life-threatening symptoms, steroids, cholestyramine washout, and biologics may be considered. Since leflunomide's active metabolite undergoes enterohepatic recycling, cholestyramine, a bile acid sequestrant, can bind and prevent its recycling and facilitate its biliary excretion instead. The utility of a cholestyramine washout has been demonstrated in cases of severe sepsis with renal failure and refractory wound healing. If the patient had not improved or presented more critically ill, this could be considered an adjunct therapy."

Reviewer B

This is an outstanding case of drug-induced colitis, a disease that is common enough to be encountered by gastroenterologists but rare enough that primary providers could miss it. The endoscopic and histopathologic images add a nice touch. I do have a few suggestions for improvement:

1) Introduction (in both abstract and manuscript) should not include details of the individual case. Give background on DDx for chronic diarrhea, explain which medications commonly cause diarrhea/colitis, etc.
   Reply 1: Thank you, both introduction sections in the abstract and main text have been edited to reflect the above comments and to include a wider DDx of diarrhea both acute and chronic. While we kept the abstract more generalized, greater detail is provided in the main text's introduction.
   Changes to text 1: Please see introduction sections in abstract and main text.

2) Need more detail of medication-induced colitis in general. Pathophysiology, common culprits, usual delays in diagnosis, etc.
   Reply 2: We have included additional information on drug induced colitis both in the introduction section and abstract. While there are many culprits for drug induced colitis each with its own microscopic and macroscopic findings we believe that going over mechanism of action and pathophysiology of each drug class would detract from the case's main topic. However, we have included more information and details on leflunomide's mechanism of action and its effect on activated T lymphocytes. We believe this adds to a more robust discussion and points towards a possible etiology of leflunomide's induction of colitis and helps contextualize our case report.
   Changes in text 2: Multiple changes have been made both in introduction and discussion sections to address this comment.

3) Change brand names for medications to generic names.
   Reply 3: Thank you for noticing this, we have revised the manuscript and made sure to replace all instances of brand names with the generic name.
   Changes in text 3: Rocephin and Flagyl are now Ceftriaxone and metronidazole.

4) The "case description" is just for presenting facts of the case. Try to move commentary on the case to the discussion section.
   Reply 4: Comments regarding elevations of lab values have been removed. We have done our best to only present facts from the case in the Case Description section.

Changes in text 4: “Complete blood count, basic metabolic panel and hepatic function panel were within normal limits. COVID-19 testing was negative. Patient had a lipase of 236, c-reactive protein of 40.7, erythrocyte sedimentation rate of 58 with a negative *C. difficile* toxin. Fecal lactoferrin was positive.”

5) I am fine with verbal consent for case reports for any disease that are not stigmatized (e.g. HIV, psych disorders, etc.). However, you should document which author obtained verbal consent.
Reply 5: Thank you for this distinction, we have fixed this.
Changes in text 5: Our ethical statement has been edited to include Kaiser Kabir as the author who obtained verbal consent via phone.

6) Several grammatical points -- you have several place in which you start sentences with "Patient" and several in which you start with "The patient." Same with utilization of the Oxford comma. Either is fine, just be consistent
Reply 6: We agree, consistency is best and we have edited the manuscript to address this.
Changes in text 6: We have opted to maintain the use of the Oxford comma and made sure sentences or references to just “patient” have been replaced with “the patient” or “this case” etc.