Peer Review File

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Reviewer A:
This is a very well-written review of ocular syphilis. However, there is nothing new in this paper.

Comment 1: Case 1. How do you explain negative RPR? (both RPR/VDRL and specific anti-treponemal tests such as FTA-ABS, or TPHA turn positive two to three weeks after infection, around the same time as the appearance of the primary lesion. See Henao-Martinez, Johnson SC. Neurol Clin Pract 2014)
Reply 1: We thank the reviewer for their thoughtful question. RPR is often negative early in infection which is when this patient first presented. No specific anti-Treponemal test was done initially by rheumatology. When they presented to ophthalmology one week later, the FTA-Abs and RPR were positive.

Comment 2: What was the time interval between the first negative RPR and second positive RPR?
Reply 2: Thank you for this clarifying question. There was one week between the first negative RPR and second positive RPR. The manuscript has been updated (lines 117-118).

Comment 3: Case 3. Was RPR or VDRL positive? Was a spinal tap done to rule out neurosyphilis in view of optic nerve involvement? Technically optic nerve is a cranial nerve and its involvement should warrant CSF examination.
Reply 3: We appreciate the reviewer's follow up questions. An RPR was performed and resulted positive. A lumbar puncture was performed, and the VDRL-CSF was negative. The manuscript has been accordingly updated (lines 134-136).

Comment 4: Line 173. VDRL or RPR alone is insufficient to diagnose syphilis due to false positives in many conditions, including vaccinations and pregnancy, and not for the reason given by the authors. (see CDC)
Reply 4: Thank you for this comment. We have included this information and specified that in addition, an RPR may be negative during primary syphilis (lines 187-189).

Reviewer B
This was a well written case review of 4 cases diagnosed with ocular syphilis in one hospital system. Ocular syphilis is one of the most severe manifestations of syphilis and can result in blindness if left untreated. The authors do a nice job summarizing symptoms and clinical characteristics of each of the 4 patients and then contextualizing these cases with the broader ocular syphilis literature for the reader. I have highlighted several places in the manuscript that need clarification, particularly in regard to syphilis testing and epidemiology. Please find specific comments below:

Comment 1: Line 28: I think most cases of ocular syphilis present in patients diagnosed with late or unknown duration syphilis, not secondary syphilis. Please see Oliver, SE et al. 2016 MMWR 2016 and Jackson, DA et al. STD 2022 for references.
Reply 1: Thank you for the correction. This has been updated accordingly in the Abstract (Lines 27-28) and in the Introduction (Lines 74-76).

Comment 2: Line 40: While probably true, I am not sure your case series provides evidence that "Early diagnosis" contributes to more favorable prognosis of ocular syphilis. It is possible that many people do not develop ocular symptoms for months or years after infection. I would rephrase to indicate that you are advocating for prompt diagnosis after the onset of symptoms.
Reply 2: Thank you for this clarifying point. The wording has been updated (Lines 40-41).

Comment 3: Line 52: You are right that the rates of syphilis are increasing faster among women, but syphilis is increasing in both men and women. Also, most syphilis continues to be diagnosed in men. You may want to rephrase this bullet point to indicate that most syphilis continues to be diagnosed in men.
Reply 3: We thank the reviewer for the clarification. This has been accordingly updated (Lines 52-53).

Comment 4: Line 81: This statement is a little misleading. Based on your reference, it looks to me that the more important (and more accurate) aspect about syphilis stage to highlight is that although ocular syphilis can be diagnosed at any stage, nearly half is diagnosed in people with late (diagnosed more than 1 year after infection) or unknown duration syphilis.
Reply 4: Thank you for pointing out this clarification of the data. This update has been reflected in the manuscript (Lines 87-89).

Comment 5: Line 112: Do you know the specific RPR titer for Patient 1? If so, please include.
Reply 5: The RPR titer is 1:128 and has been included (Line 118).

Comment 6: Line 113: Why was this patient not treated with the recommended treatment for ocular syphilis (IV penicillin for 14 days)?
Reply 6: The decision to treat the patient with IV ceftriaxone instead of IV penicillin was at the discretion of the infectious disease doctor. Unfortunately, we do not have further rationale available.

Comment 7: Line 127: Did patient 2 have any non-treponemal test results? If so, please provide. Include RPR titer, if available.

Reply 7: An RPR was performed but unfortunately the titer is unavailable. The RPR positive results and the results of the lumbar puncture (VDRL-CSF negative) have been added accordingly to the manuscript (Lines 134-136).

Comment 8: Line 140: Did patient 3 have any non-treponemal test results? If so, please provide. Include RPR titer, if available.
Reply 8: We have included that the RPR was positive; however, we unfortunately do not have the titer available (Line 151).

Comment 9: Lines 165-167: Are you stating that it is possible that more cases could present in women currently? I think you need to clarify and specifically state this point. Providing information about the percent change in syphilis in men during 2016-2020 as well as the prevalence of ocular syphilis by gender (one estimate published by Jackson, DA et al. STD (2022)) may help support this idea.
Reply 9: Thank you for your comments to clarify the point. We have included this additional source as well (Lines 177-181).

Comment 10: Line 173: Treponemal testing alone is also problematic. A positive treponemal does not necessarily identify a new syphilis infection. It may be detecting an infection that has already been diagnosed and treated. Please emphasize that both treponemal and non-treponemal testing is needed to diagnose syphilis.
Reply 10: Thank you for this feedback. The importance of both treponemal and non-treponemal testing has been re-emphasized (Lines 187-189).

Comment 11: Line 195-196: Can you comment on differences in symptoms (e.g., severity) or treatment resolution by HIV status? Also, you need to rephrase that the recommendation is that "all patients not known to be living with HIV be tested for HIV." There is no need to test a person who has already received a diagnosis of HIV. For these people, it's important to assure they are receiving care and treatment for their HIV.
Reply 11: We appreciate the feedback to include more data on syphilis with HIV stratification. The recommendation has been appropriately edited both here, the abstract, and in the conclusion (Line 39; Lines 210-218; Lines 244-245).

Comment 12: Line 224: As mentioned in the abstract, while this statement is probably true, I am not sure your case series provides evidence that "Early diagnosis" contributes to more favorable prognosis of ocular syphilis. It is possible that many people do not develop ocular symptoms for months or years after infection. I would rephrase to indicate that you are advocating for prompt diagnosis after the onset of symptoms.
Reply 12: Thank you for this clarifying wording. It has been reflected in the Conclusion (Line 246).

Reviewer C

Introduction:

Comment 1: line 71: it would be useful for the readers to indicate which proportions attain the rise of cases in the US.

Reply 1: Thank you for this feedback. We have included that most of the rise is attributable to late or unknown duration syphilis (Lines 74-76).

Comment 2: line 73: it would be better to get a short introduction (in 1-2 sentences) about the different stages of syphilis.

Reply 2: We have now included a brief description of the different stages of syphilis (Lines 71-73).

Comment 3: line 80: it would be preferable to introduce the statistic of ocular involvement, including the proportion as the first presentation of syphilis infection in the introduction than in the discussion at line 162.

Reply 3: Thank you for this feedback. We have moved the statistic of ocular involvement from the discussion to the introduction (Lines 81-82) and added an additional sentence to describe the proportion of ocular syphilis as the initial presentation (Lines 82-83).

Comment 4: line 84: please define low visual acuity

Reply 4: We have clarifier that it is a "lower" visual acuity, as in relative to the other patients in the study, rather than a true objective low visual acuity cutoff (Line 91).

Comment 5: line 90: introduce the abbreviation

Reply 5: *AME Case Series* is the accepted title of the Checklist and is not commonly written out. We have clarified by italizing the name (Line 100).

Comment 6: line 122: which symptoms?

Reply 6: We have clarified that there were no systemic symptoms accompanying the ocular complaint (Lines 128-19).

Comment 7: line 126: introduce the abbreviation

Reply 7: We have now included the long form of the abbreviation (Line 132).

Comment 8: Patient 2: Was there no non-treponemal test being carried out?

Reply 8: Thank you for this follow up question. An RPR was performed but unfortunately the titer is unavailable. The RPR positive results has been added to the manuscript (Lines 134).

Comment 9: Patient 3: A woman with unilateral optic neuritis (ON) does not lead to the first-line suspicion of ocular syphilis. Do you have carried out a classic work-up for ON? Once again: Was there no non-treponemal test being carried out to assess the activity?

Reply 9: Thank you for your comments on the additional workup for optic neuritis. We have included the results of her MRI Brain, as well as the pertinent other serologic workup (ACE, lysozyme, QuantiFERON). We have also included the positive RPR. (Lines 148-152).

Comment 10: It lasts weeks before an optic nerve oedema resolves. When the reader read your article, she/he can get the impression that the oedema resolves after 14 days, which is improbable. After which period of time did you perform the control?
Reply 10: Thank you for this clarification on the timeline. We have updated the manuscript to say that at her subsequent follow up appointment the edema was improving but not yet fully resolved (Lines 153-154).

Discussion:
Comment 11: it would be useful for the reader to get information about the prevalences and incidences of the different differential diagnoses to be able to assess how frequent/rare and thus how probable it is to face a syphilis infection when confronted with ocular inflammation. Hence, ocular syphilis was and remains, despite the recent increase a rare affection.
Reply 11: Thank you for this comment. We have emphasized at the start of our discussion that ocular syphilis, while important, is a rare disease entity. (Line 173).

Comment 12: line 205-206: why was a patient treated with ceftriaxone and not penicillin?
Reply 12: The decision to treat the patient with IV ceftriaxone instead of IV penicillin was at the discretion of the infectious disease doctor. Unfortunately, we do not have further rationale available.

Comment 13: Do you have taken any preventive measures for the Jarish-Hexheimer reaction?
Reply 13: We do not take preventative measures but supportive care and counseling on the side effects are provided.

Comment 14: General consideration:
- The article is globally well-written and treats about a currently important topic.
- At moments, we wish to have more details of one or another case as well as an equally structured presentation of the cases:: symptoms, clinical findings, workup, treatment and outcomes.
- These four patients are the only ones in this period of time at your centre? If not, why did you choose them particularly? Just because of the coverage of different clinical presentations?
- The potentially most important finding is the high proportion of women. It is the best "appetizer" in the introduction. It does not appear at all in the discussion. It can maybe be part of the review to see if there are other studies that find a discrepancy or a recent

change in the epidemiologic profile of patients with ocular syphilis.
- There is a lot of general information about the disease that is to find in the discussion instead of the introduction. Is that a requirement of the editorial board?
If not, I would find it better to introduce the disease and the important facts in the introduction instead of in the discussion.

Reply 14: Thank you for these general consideration points. The four cases were chosen to demonstrate various clinical presentations of ocular syphilis. This is reflected in the introduction as a non-consecutive case series and we added that these four cases were selected for such purpose (Lines 94-96). We have also included additional discussion on the rise of ocular syphilis in women (Lines 177-181).