Author’s response to reviews

Title: INCREASED INCIDENCE OF SUSAC SYNDROME: A CASE SERIES STUDY

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Author’s response to reviews:

We thank the reviewers for their comments. We have revised the manuscript based upon the reviewer’s comments and marked changes in red in the revised version.

Reviewer 1
This is a well described case series of patients diagnosed with Susac syndrome. I have a few comments that should be addressed in a revised version:
Diagnostic procedures:
1. Did patients receive spinal cord MRI? Data on potential SC involvement is scant so it would be interesting to learn more about SC affection if available
Unfortunately only one of our patients (patient number 6) had a cervical spinal cord MRI done at disease onset. No evidence of cervical spine involvement was detected.
2. Was OCT done? This is also an emerging diagnostic tool showing very specific changes in SuS. If not, this should be mentioned as limitation and discussed in light of the pertinent literature, see PMID: 26200513, PMID: 26203089
We thank the reviewer for shedding light on this important aspect of diagnostic workup in Susac Syndrome and have included the suggested references as well as a description of the findings on OCT in this group of patients.
Spectral-domain optical coherence tomography (OCT) was done in all cases. Macular OCT scans were evaluated for (1) areas of hyperreflective thickening of retinal nerve fiber layer to the outer plexiform layer which is indicative for tissue swelling due to acute branch retinal artery occlusion (BRAO), and (2) areas of thinning of these layers indicative for previous ischemic damage. Three patients showed signs of acute retinal hypoxia indicating on an acute macular BRAO. Additionally, five patients showed signs of macular retinal thinning, compatible with previous events of macular ischemia. Two patients had normal OCT.

Results / findings:
3. CSF should be added to the table for each individual patient, this is important as CSF changes in SuS have not been thoroughly investigated.
Thank you, this is indeed an important note and we have revised Table 1 accordingly to include CSF parameters.
See Table 1
4. Brain biopsy: in which region of the brain was this performed, leptomeningeal?
One of our patients (patient number 6) who presented with severe encephalopathy underwent stereotactic brain biopsy from an active lesion on his right parietal lobe. The samples include fragments of cortex, white matter and some vessels from subarachnoid space. There was no evidence of vasculitis or inflammatory reaction. CMV immune-stain was negative.
See results section, diagnostic procedures, page 9, paragraph 4.
5. Please report whether there was callosal atrophy or hypointense callosal lesions in any of your patients, this is another characteristic imaging feature, please see and add PMID: 22711711. This feature can also be detected at lower field strengths.
We agree with the reviewer's comment regarding the importance of both callosal atrophy and callosal hypointensities as characteristic features of this syndrome and have included the suggested reference as well as a description of these findings the cases series presented.
Of note Corpus callosum hypointense T1 lesions were found in all our patients and corpus callosum atrophy was evident in five patients.
See results section diagnostic procedures, page 8, paragraph 1.
6. Did you assess lesion evolution over time on follow-up MRI? It would be interesting to learn if lesions disappeared or remained constant, if atrophy of the CC and the entire brain progressed-the latter can be assessed non-quantitatively on FU scans.
We accept this suggestion and have revised the results section in the manuscript to include data regarding follow up MRI scans:
Brain MRI lesions remained unchanged in five patients however, in one patient some lesions disappeared in follow-up MRI. Follow up MRI revealed Global brain atrophy and corpus callosum atrophy in four patients.
Discussion:

7. See above on OCT plus refs - should be touched upon
   We have included OCT and the suggested referenced in the discussion.

8. What do the authors believe is the reason for the increase in incidence of SuS?
   The reported observed increase in Susac Syndrome diagnosis in our center drove us to believe it is important to bring this rare condition to the attention of physicians and led us to explore an explanation for this increased incidence. Our attempts did not result in a definitive conclusion but we have found some evidence supporting recent infection which we have reported.

9. Were patients re-tested for CMV upon FU? Could also briefly be mentioned in the results section
   CMV serology was retested in two patients; in one patient there was evidence for seroconversion from IGM to IGG and in the second patient both IGG and IGM levels remained high during follow-up, this patient showed clinical and laboratory evidence for CMV reactivation.

10. Treatment of SuS: important to mention that MS drugs may worsen / exacerbate, please see and add PMID: 26445727
   This is indeed in important comment and we have included this clinical note and the suggested reference to the discussion.

Reviewer 2:

The manuscript „Increased incidence of SUSAC syndrome: a case series study" by Wilf-Yarkoni et al presents a single-center retrospective study on the incidence of Susac syndrome (SuS), a rare immune-mediated occlusive microvascular disease, in patients in the Surasky Medical Center in Tel Aviv, Israel. The authors report on 7 cases diagnosed and treated in their center from July 2017 to August 2018.

The main findings are (i) an increased incidence as compared to the reported incidence in a study from Austria and (ii) a putative correlation to infectious diseases as insinuated by positive CMV IgM antibody titers in 3 patients and increased anti-Streptolysin titer in one patient. Based on these observations, the authors conclude that it is worthwhile searching for a post-infectious state in newly diagnosed SuS patients as well as to screen for latent infections.

General critique:
This is an interesting case collection in a single center retrospective study, which highlights SuS as a rare yet important disease with therapeutic consequences in affected patients. Indeed, bringing this rare condition to the attention of physicians and translationally minded scientists is of great importance, which in this reviewer's opinion constitutes the main value of this manuscript.

That said, severa limitations on the study itself as well as the conclusions drawn emerge, which should be addressed in a revised version of the manuscript.

1. Most importantly, it is hard to accept the conclusion and supposed main finding of an increase in incidence based on the data presented. Comparing cohorts from another country with varying demographic and ethnic background may not represent the best way to conclude on an increase in incidence. Instead, could the authors present numbers for the last years of their center, maybe present them in a graphical yearly form to calculate the significance of their finding?
   This is an important comment which, unfortunately can only be partially addressed since no local registry exists and hence we can only rely on previous case series published (Vishnevskia-Dai, V., et al., Susac syndrome: clinical characteristics, clinical classification, and long-term prognosis. Medicine (Baltimore), 2016) or registries collected in other countries (Seifert-Held, T., et al., Susac's syndrome: clinical course and epidemiology in a Central European population. Int J Neurosci, 2017.) to quantitatively assess increase in incidence.
   We agree that adding to the text and graphically the incidence of SuS in our single center may highlight the significance of this observation and have included this data to the results section of the manuscript as well as in a graph as suggested.
   Two patients were diagnosed in our medical center from 2013-2016– one patient in 2013 and one in 2016, therefor our case serious represents a 7-fold increase in the annual incidence expected in our medical center.
   See abstract section, results page 2; introduction section page 4, paragraph 3; results section, Clinical characteristics, page 6, paragraph 1; discussion section page 11, paragraph 1.
   See supplementary, figure 1.

2. Moreover, reasons for this potential increase in SuS incidence should be discussed (e.g. diagnostic criteria) in a revised version of the manuscript.
   The reported observed increase in Susac Syndrome diagnosis in our center drove us to believe it is important to bring this rare condition to the attention of physicians and led us to explore an explanation for this increased incidence. Our attempts did not result in a definitive conclusion but we have found some evidence supporting recent infection which we have reported.
   It is important, as the reviewer suggests, to recognize potential confounders such as a rise in awareness due to recently published diagnostic criteria and suggested therapeutic guidelines (Rennebohm, R.M., et al., Guidelines for treatment of Susac syndrome - An update. Int J Stroke, 2018: and Kleffner, I., et al., Diagnostic criteria for Susac syndrome. J Neurol Neurosurg Psychiatry, 2016).
   However, this publications did not cause a shift in diagnosis as criteria have not been revised.
   We have revised the manuscript to include this potential confounder.
   See discussion page 13 paragraph 8.

The authors point out in their discussion that there is no known correlation with infectious diseases and the occurrence of SuS. Yet, they postulate an infectious etiology due to IgM serum titers for
CMV and increased anti-Streptolysin titer. However, this might be a mere co-incidence and in no way causal.

Especially, did the authors measure IgM antibody titers in the CSF of these patients as well? CMV IgM antibody in the CSF were not measured, only PCR for CMV was tested as well as CMV immune-stain done on tissue obtained from brain biopsy.

See results section, diagnostic procedure page 8, paragraph 3; discussion page 13, paragraph 8.

Did the IgM antibodies in the serum convert to IgG over time?

CMV serology was retested in two patients; in one patient there was evidence for seroconversion from IgM to IgG and in the second patient both IgG and IgM levels remained high during follow-up, this patient showed clinical and laboratory evidence for CMV reactivation.

See results section, diagnostic procedure page 8, paragraph 3; discussion page 13, paragraph 8.

Were CMV PCRs done in the serum/PBMCs as well?

CMV PCR was done in the CSF. CMV PCR in the serum was done for two patients; patient number one had 543 IU/ml and patient number four had 70,635 IU/ml and was negative after treatment with valacyclovir.

See results section, diagnostic procedure page 8, paragraph 3; results section, treatment regimen and clinical outcomes, page 11, paragraph 3.

In these lines, were there increased anti-Streptolysin titers in the CSF as well?

We did not measure anti-streptolysin titer in the CSF.

See results section, diagnostic procedure page 8, paragraph 3.

Providing these data would be important to postulate an infectious etiology. If not, for sure the limitation of their conclusion (co-incidence, false positives etc) should be discussed in more detail in the discussion.

We accept this comments and are aware that due to the retrospective nature of the study, data was not collected in complete. This limitation is fully acknowledged and mentioned as a limitation of the study in the discussion.

We have changed the wording in the manuscript to clarify that the evidence connecting infectious etiology and Susac syndrome are weak and that this could only represent co-incidence / false positive. However, we believe that sharing these observations is important to raise an awareness to this possibility for others to further explore.

See discussion section page 13, paragraph 8.

3. Did the authors check for serum and CSF anti-endothelial cell antibodies, a potential etiology of SuS brought forward in the discussion?

Anti-endothelial cell antibodies were not checked. Although high levels of anti-endothelial cell antibodies has been reported, titers >1:100 were found in only 25% of patients with SuS therefore, are not included in the diagnosis criteria of SuS. (Jarius et al. clinical, paraclinical and serological findings in susac syndrome: an international multicenter study. J Neuroinflammation 2014 Mar).

See discussion section page 13, paragraph 5.

4. Since one potential aim of this report may be to increase the awareness about this rare disorder, it would be appreciated if the authors explained the standard diagnostic criteria and treatment regimen in more detail. A table based on the recent criteria might be useful to achieve that aim.

We added supplementary tables describing the diagnostic criteria (Supplementary, Table 1) and treatment regimen according to CNS severity (Supplementary Table 2).

Minor points:
5. Please define abbreviations when first using them, e.g. BRAOs p.8, line 8
   Abbreviation for BRAO was added when first described on page 5.
   See methods section, Study design and data collection, page 5, paragraph 4.

6. Please use complete sentences (p.9, line 5)
   Thank you for your comment, we have changed the sentences accordingly.
   See results section, Treatment regimen and clinical outcomes, pages 9, paragraph 1.

7. Table 2: please use generic pharmaceutical names instead of brand names (aspirin)
   We have changed all the brand names in the table to the pharmaceutical names.
   See table 2.