Reviewer reports:

Reviewer #1: MSC treatment has been applied in many large academic centers in an experimental setting for GHVD and increasingly for SLE and JIA. The present manuscript presents 3 pediatric cases where the disease appeared to be resistant to conventional therapy. Then something unorthodox was done: patients travelled to far away clinics (cancun Mexico;AMA Regenerative Medicine & Skincare; Stem cell institute Panama)) were MSC were given, almost as if it were an over the counter product. Onlu cell number per kg were noted. None of these centers is present on the co-author list. The infusion regimen in patient 1 includes intranasal injection, lymphnode injevtions and i.v. (4 dosages). Such injection methods should have been reviewed by an IRB together with a medical protocol. The lack of this is unacceptable.

The background provides inadequate overview of the litarature. Data on myocardial infarction cannot be viweed as promising because there are many studies failing to show an effect. There is a lot of recent SLE literature that is not cited. As there is also a study on 6 paediatric cases with JIA (Rheumatology (Oxford). 2019 Oct 1;58(10):1812-1817. doi: 10.1093/rheumatology/kez157.). Furthermore the issue of autologous vs allegeneic is not discussed at all.

Case 1 is SLE without nefritis that is doing well (F.U 18 months). Case 2 pursued her own MSCs from a biobank. She was given iv and local intra-osseous injections into the femoral bone lesions. The course of the femoral lesions remains unclear, a scheduled hip replacement was cancelled. Severe osseous lesions cannot be expected to be cured by local MSC injections. MRI documentation is lacking. Case 3 is an RF pos JIA, treated with MTX and Enbrel when an MRI showed synovitis and new erosions. Because of severe pain in the elbow the patient and her parents went to Panama where she received 3 infusions over 4 days (umbilical cord derived). MRI 5 months later showed progression. It is noted that RF remained positive (which is not relevant) *(removed from text)*, then after new MSC infusions she received intra-articular steroid injection with good result. The family then chose for another holistic therapy.

Response to Reviewer 1

There were two purposes for writing this paper: the first is that these cases are done in a random, uncontrolled fashion emphasizing the need for well thought out and controlled clinical trials and the second is that there appears to be a positive response from the therapy that fortifies the need for clinical trials. Yet, our patients sought these institutions out that provided therapy without proper controls. None were listed as collaborators since

our physicians only found out about the therapy after it was done. None of them contacted us or asked whether the patient had any special needs or concerns. In the initial paper, we included the names of the institutions, but took out these names since there were not authors or contributors *(deleted in various pages)*.

Edits made include multiple sections for increased detail (Page 1, 2). Included comment on MSC potentially leading to pro-inflammatory process (Page 4, new reference 5, Page 15-16). Changes to show that authors were not in agreement with MSC therapy and had made no contact with the MSC transplant team were added for case 1 (Page 6), case 3 (Page 11). Defined the conclusion to make sure the readers understand the main point (Page 17-18).

To be clear, these cases were presented since these families lost faith in traditional medicine and sought expensive care elsewhere. For case 1, she continues to be well and had required multiple therapies including methotrexate and azathioprine to control her disease. She is now off medication. While it is true, she never had renal involvement, she had severe arthritis. This resolved post-MSC transplant based on our clinical exam. The second case had severe toxicities from medications. She developed myositis and avascular necrosis, as shown in the MRI figures now included. While it is not clear how MSC could prevent femoral head collapse, this is what we have observed, and she is back to dance without pain whereas she was bedridden before transplant. The third had seemingly the least benefit requiring an elbow injection after the transplant, but patient and parents report she is improved and are happy they sought out the MSC transplant. These cases suggest there may be a benefit to this therapy that should be studied as an adjunct to our current therapies.

MRI image of AVN and myositis added for case 2 on page 10

Reviewer #2: "Since MSC research remains a political, cultural, and religious area of contention, the breadth of research, especially in the pediatric population, is scarce within the United States."
Mesenchymal stromal cells are adult stem cells that can be derived from fat tissue or bone marrow. I don't know why that would be a political, cultural or religious problem. Stem cell transplantation with hematopoietic stem cells are performed world wide without problems as well.
Mesenchymal stem cells are quite heterogenous. If somebody wants to publish about it one should be able to state exactly the release criteria (including viability of the cells) and how it was defined that they are true MSC.
From the article:
Case 1: The administered autologous AT-derived MSC doses given are huge (normally it is 2

million per kg iv) and since they give it via 3 routes in case 1 it seems like they are just trying something without a clear idea. The recurrent episcleritis is as the name suggest a fickle symptom, like lupus as a disease which makes it really hard to tell for 1 case whether a treatment is effective. We are not informed on when the last biological was added to the regimen (since this has been continued) and whether any effect seen could be due to that biological. Apparently after the MSC treatment (which was in a 9 month time frame) she had a transient flare with arthralgia, malar rash, and recurrence of scleritis, which subsequently resolved in 2 months without treatment.
Case 2: She was started on tocilizumab and received four doses administered intravenously every 14 days with improvement, but also pursued local injection of allogeneic Wharton's jelly MSC. Again no release criteria or any rationale for doses or administration routes: Three hip injections (1 cc of Wharton's Jelly MSC; 1.1 x 106 MSCs) were given at months 0, 2, and 6, while intravenous transfusions (60 x 106 MSCs; 1.2 x 106 MSCs/kg/dose) were given at months 2 and 6. It will be really hard to claim that the MSC were the reason that her femoral heads did not collapse and therefore she did not need surgery (yet) while she started tocilizumab at the same time and the X-rays look the same.
This sounds like wishful thinking.
Case 3: routine MRI of her elbow then showed active synovitis and new erosions, which led to a recommendation to switch to another biologic therapy. However, severe localized pain with biologic injections prompted the family to
explore stem cell therapy abroad. She transferred back to our institution due to disagreement about MSC therapy with the outside institution. The transplant physicians instructed the patient to wean off her rheumatic medications prior to the transfusion. I am not so sure that the institution of the authors did the right thing to go along with it and I can understand the outside institution for their objecting to a non-study treatment which will be another n=1 case that can not be compared to anybody else. An MRI of her left elbow 5 months post-transplantation revealed continued active synovitis and progression of erosive changes. The family however continued their desperate search apparently: a complement of holistic management including monthly hyperbaric oxygen, red-light therapy with in-home lymphatic draining twice weekly, and daily pulsed electromagnetic field therapy. Of course we can not stop 16-year olds and their family from trying non-proven therapies, but I do
think that as treating physicians we do have the obligation to tell them that it might harm (time, energy, hope, financially) and I could not find such a note in the results although it was mentioned in the discussion that it was always the sole decision of the family.

"All cases: Although some mild adverse effects were endorsed, all patients felt
that MSC treatment was beneficial in achieving disease remission with drastic improvement in patient global assessment."

This is called the placebo-effect combined with the reporting bias. A recurrent episcleritis can not be called a success due to MSC since it is recurrent by nature.

The authors state: The decisions to adopt MSC treatment was difficult since stem cell research is lacking in the field of pediatric rheumatology. They state there is a lack of studies, but apparently were missing out on the exisiting ones e.g. (doi:10.1093/rheumatology/kez157 JF Swart etal. ; 10.1155/2016/9165267 Wang L etal.).

The authors assembled cases from which they heard people went to such a center for treatment with MSC without any decent treatment protocol or ethical consent for a study (on the way to creating a protocol).
All three cases presented in this report demonstrated years of poorly controlled disease unresponsive to multiple non-biologic and biologic DMARDs prior to initiating MSC, which makes these people completely desperate and looking for alternative treatments. All similar patients that also went to such centers and did not experience any benefit not even a lasting placebo-effect, but felt ashamed that they paid so much for a con job will likely not tell their physician. This is how alternative medicine gets its free advertisement. If you really want to publish about any effect and stay away from this reporting bias, one should first do an anonymous inquiry on their patients to see how many really went to these procedures.
In fact I believe this is an industry (the authors indeed state commercial clinics) in which they benefit from not having randomized controlled trials or trials (with homogeneous protocols) at all, since people apparently come to them even though they did not show efficacy, nor will they ever be able to with all different kinds of MSC sources, dosing regimens and administration routes. Being licensed to administer cells should not stand in the way of examining if cell treatment really works, which should come first in my opinion.
If the title would run "Mesenchymal stromal cells in desperate patients, the downside of commerce in medicine" and then state all the physical (lymph node injection, 3x ia injections, iv infusions, the risk these "transplant" physicians take with advising children to wean off immunosuppressive agents while likely having no knowledge of autoimmune diseases) and financial (and emotional) harm they placed on their patients the topic would be more appealing for our readers. In fact in the discussion the authors already specify: One family sold their car, and a second family re-mortgaged their home in order to pay for the treatment they felt was necessary to alleviate their child's rheumatic disease. That should be the main focus of this article and how to stop others from going down that same path instead of encouraging people to try to get the same placebo-effect ("This retrospective series highlights the potential of mesenchymal stem cell therapy in refractory pediatric rheumatic diseases").

Response to Reviewer 2

Although is true that MSC transplants are approved in many countries around the world, they are not approved in the US for therapy of autoimmune disease. In Canada, they are approved for GVH disease, but not autoimmune disease. Our premise is that clinical trials should be done in the US in this patient population with rigorous controls to validate whether or not MSC transplant therapy is of value.

Again, re-written to highlight further research, development of standardized infusion therapy protocols and well-designed clinical trials as essential. Page (1, 2). Also mentioned in the very beginning of Discussion and Conclusion on page 12.

In all of these case, our institution did not go along with it. In case 1 and 2, there were questions, but in none of the cases did we suggest they pursue this therapy and to our dismay, the treating institutions did not contact us or inform us of our patients' plan to seek therapy. We learned of it after the MSC-transplants in all cases. In case 1, the reason for the routes given is not clear since the institutes offering these therapies did not seek our advice or offer explanations for their preparation of MSC or the preferred routes of therapy. The MSC transplant did appear to have benefit since she was weaned off all immunosuppressive medications (azathioprine and previously methotrexate were stopped prior to transplant) and only Plaquenil was continued for about a month. She still uses ocular steroid, but otherwise is off all medications. In case 2, as in case 1, no one from the institute consulted us. She did much better after the transplant since she was bedridden and afterward was able to return to school and dance. She continued conventional medications for several months, but then weaning off all. She did well for about 2 years, but now is developing increased disease activity. She and her family refuse conventional therapy and are saving up for a repeat MSC-transplant. In case 3 it is not clear she benefitted, but as the reviewer points out, there may have been a placebo effect, which we agree may have played a significant factor.

We agree with the reviewer that this clandestine therapy without consulting the treating physicians should be stopped. We changed the name of our paper as the point is well taken, but did not feel the direction of the name change was appropriate *(Page 1)*. The cost should not be born by the family and if the trials are effective, insurance should be involved in covering the cost. We also updated the discussion to include multiple recent publications.

Reviewer #3: Very interesting presentation of three patient cases and treatment modality needing to be explored in much more details. It is unclear what was the post MSC transplant follow up time for each of the cases so could authors provide this information.

Response to Reviewer 3

The post-MSC transplant is presented in our text and tables (newly added).

Reviewer #4: Thank you for the opportunity to review the manuscript titled: "Mesenchymal stem cell treatment for refractory pediatric rheumatic diseases: A single center case series".

Regarding the same I have the following comments.
1. For the measurement of the results with the suggested treatment, only the global assessment of the doctor and the patient is available. Don't they have other more objective measures? The way they assess response to treatment is highly subjective. Without these objective measurements it is not possible to evaluate the effectiveness of the therapy

Response to Reviewer 4
Since our institution was not involved in the MSC transplant, there is not planned pre- and post-MSC therapy clinical parameters, but we did our best to include them in our updated paper with table addressing non-subjective findings.

Added SLEDAI-2K scoring on page 6-7, SLEDAI-2K on page 8-9, and JDAS score in table on page 12
Added disease activity scoring in Summary Table on page 12

Added Myositis activity labs (CK, aldolase, LDH) in case 2 on page 8

Reviewer #5: The authors have presented 3 cases who received varied forms of MSCs and showed some benefit in control of disease. Though it is an interesting piece of information it has major issues

1. The type of MSCs used are variable with different doses, different time points
2. No use of validated disease activity measures like SLEDAI/NILAG for SLE, JADAS for JIA or 6 minute walk time, pulmonary function test or CT chest for MCTD
3. Uncontrolled study with lot of additional drug use
4. Non use of standard therapies like cyclophosphamide, RTX for SLE

Thus it does not add much. It may be suitable as a letter just to suggest that MSCs may have a benefit in such situation with a short discussion

Response to Reviewer 5:

-Noted previously, the physicians were not aware of the plan to undergo MSC therapy and were not involved in the MSC type, preparation or infusion of MSCs in these patients.

-We did a retrospective disease activity measure in the first two cases. The third patient went on to seek out holistic medicine.

Added SLEDAI-2K scoring on page 6-7, SLEDAI-2K on page 8-9, and JDAS score in table on page 12
Added disease activity scoring in Summary Table on page 12

-Of note, on our review, often the patients did not tolerated or were fearful of the drugs and did not give the medications a trial without stopping or requesting to switch medications, refusing to try the medications for longer.

-In case 1, the patient with SLE, she was on Imuran and did not have renal disease necessitation the use of Cytoxan. In case 2 with MCTD, Benlysta was effective for some time, but the patient decided it was not working and quit the medication. The physicians felt the Benlysta was working well.

Reviewer #6: Article is of interest as a not well known option for patients with broad range of rheumatic disease described in the case series. It raises awareness of treatments on the horizon to be on the lookout for.

Response to Reviewer 6:

This was a main purpose of our paper.

Reviewer #7: The experience reported by Wong et al. on the use of MSCT in children with different rheumatic diseases is interesting, even if several concerns could be addressed.

1) Authors could cite also the limited data available on RA. Karamini et al (Stem Cell Rev and Rep 2020; 16: 276-287) systematically search and review the literature for randomized or non-randomized clinical trials, he underlined that "...modest improvements were found in RA symptoms and RA-related indices. ... clinical response criteria related to RA were achieved by a low-to-moderate percentage of patients. ... treatment of RA with MSCs appears to have a short-term therapeutic effect"
2) more information are welcomed on the use of MSCT for the treatment of avascular necrosis, since the patient 2 was treated with intraosseous injection of MSCs
3) Standardized measures of disease activity should be reported for all the patients and the time of follow up should be mentioned
4) Authors should justify the reason with patient 3 was not treated with adalimumab before

to explore the MSCT, since adalimumab presents a good response rate in chronic arthritis B27 related.

Response to Reviewer 7:

-The discussion has been updated to include the articles suggested and several very recent articles have been added.

Multiple (10) new references added including Karamini et al on page 5, reference 9. Also, new references 10 and 11 in Discussion and Conclusion on page 13. All new references added and italicized on paged 20-25.

-Case 2 continues to do well from the standpoint of AVN, though after 2 years she is starting to have more disease activity post MSC transplant. The response to the interarticular MSC injection is worth noting and deserves further investigation.

-In the current paper, we addressed disease activity and length of follow up in the text and tables. *See previous pages of additions regarding DAI and table.*

-This point is well taken and was because the patient refused injections.