Peer Review File

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Reviewer A

Comment 1: Please state/clarify the aim of the study despite there was a hint of that in page 6 lines 9-11.

Reply 1: The purpose of this study is added in the text.

Changes in the text: The purpose of this study is to evaluate the efficacy and safety of sclerotherapy with bleomycin based on pre- and postprocedural clinical data and magnetic resonance imaging (MRI) examination.

Comment 2: Please state if this was a retrospective or prospective observational study. I presume it was retrospective.

Reply 2: The study was a retrospective surgery.

Changes in the text: This retrospective study included 46 patients who were diagnosed as large diffused microcystic LMs (>1% body surface area) between January 2012 and December 2019 according to the medical history, imaging, and clinical manifestations.

Comment 3: Were all patients with large diffuse microcystic lymphatic malformations treated with bleomycin only, or were there any patients who were treated with other sclerosants including mixed with bleomycin? If yes, how many, and how did the authors decide on who should be treated with bleomycin / other sclerosants?

Reply 3: All the patients in this study were diagnosed as large diffuse microcystic lymphatic malformations, which was >1% body surface area, and treated with bleomycin only. We chose bleomycin as the first-line management on microcystic LMs in our unit according to the reports of effect and side effects of sclerotherapy with other sclerosants. Bleocymin owned higher complete solution rate and lower complications. The reasons of choosing bleomycin has been showed in the Discussion section.

Changes in the text: No changes were made in the text.

Comments 4: The outcome measures were mainly the radiological change of size of the LM, and adverse events. Could the authors provide other clinical measures like infection rate, pain score, and quality of life?

Reply 4: There was no infection after the treatments, and the pain in and after the treatments was so mild that no patients complained it, because the large diffuse microcystic LMs in our study were mostly shown in the skin without involving deep tissue, and the injection methods we used was minimal invasive. The results have been shown in the Results section. The main symptom of the disease influencing the quality of life is lymphatic fluid leakage. In our study, six patients had lymphatic fluid leakage, and after the treatment, it disappeared completely. We have added this data in the Results section.

Changes in the text: In the first paragraph of the Results section: Six patients had lymphatic fluid leakage which was the main symptom of influencing the quality of life, whereas the remainder complained of focal swelling and concerns regarding appearance. In the second paragraph of the Results section: The lymphatic fluid leakage was effectively cured after 2-3 courses and the quality of life was improved dramatically.

Comments 5: In my opinion, post-operative localised swelling is part of the natural process of the treatment rather than adverse event. What do the authors feel about this?

Reply 5: We listed postoperative localized swelling as one of adverse events because most of the patients after sclerotherapy showed very mild swelling in clinic, but the 7 patients in our study showed relatively obvious swelling. The description in our study was not precise enough, and we made revision about it.

Changes in the text: One of the most common adverse events was evident postoperative localized swelling, which subsided in 3 to 5 days, and showed in 7 patients (15.2%). We listed it as one of adverse events because most of the patients after sclerotherapy showed very mild swelling that could be ignored.

Comments 6: Sirolimus treatment is fairly effective in some of these LM cases. Do the authors have any experience on sirolimus including to complement the sclerotherapy treatment of LM? Please comment.

Reply 6: Sirolimus is an immunosuppressor. It is always used in the treatment of complex LMs as its antiangiogenic and antiproliferative properties through oral administration. Our study mainly discussed sclerotherapy by local injection, therefore, we did not add relevant comment in the Discussion section. In 2019, a clinical trial reported that 0.1% sirolimus cream was effective on treating cutaneous microcystic

lymphatic malformations, providing a noval therapy. We did not have any experience with sirolimus, and it could be used in our further clinical treatment and compared with bleomycin to search for a better therapy.

Changes in the text: There was no change in the text.

Comments 7: Finally, there already many reports on the use of bleomycin in treating LM in the literature. Please could the authors comment, perhaps in the Discussion, what are the novel findings and/or how this article contribute to improve the readers' practice on treating LM.

Reply 7: We have added it into the Discussion section.

Changes in the text: Until now, few published studies have evaluated the objective response in the treatment of bleomycin on large diffuse microcystic LMs. Our study, for the first time, demonstrated that the combined methods of intralesional lavage and intradermal injection were effective and safe to treat large diffuse microcystic LMs with low risk of complications and negligible lung toxicity. The success rate was 92.4% (excellent and moderate results), which was similar with the previous reports about bleomycin sclerotherapy in microcystic LMs.

Reviewer B

To refine this report, the way of intralesional bleomycin lavage should be described more in detail. According to the article, the authors punctured into all the large cysts. You should clarify the definition of "the large cysts" because the candidates are microcystic, not mixed. In addition, how many points did you puncture at a single session on average? Did you change the entry point at the next session? How did you decide to stop the sclerotherapy? In my opinion, how to do the sclerotherapy is the key point of this article because it directly affects the result.

Reply: The details have been added into the text. The large cysts were the ones whose diameter was >5mm in the lesion.

Changes in the text: (1) It was better to aspirate the overflowed lymphatic fluid to optimize the effect. To minimize lung toxicity, intralesional bleomycin lavage was performed between the two scalp acupunctures at a concentration of 1mg/ml at a flow rate of 1ml/min in the larger cysts whose diameter was > 5mm detected by ultrasonic inspection and palpation (Figure 1). (2) Four-to-6 lavages were used into the larger components of the microcystic lymphatic malformations.

(3) At the next session, the entry points were not changed because the location of larger cysts were same.

(4) We assessed the final result of sclerotherapy on the basis of a multidisciplinary consensus during the patient's follow-up visits, considering the objective and subjective results at each visit.

Reviewer C

Comments 1: For example, it is not clear to the reader why the two presented cases are outlined with greater details. Data presentation can be improved in general.

Reply 1-1: The two cases presented were typical examples of the treatment. We believe it could make a deep impression on the readers with the help of these concrete images and detailed illustrations.

Changes in the text: There was no change in the text.

Reply 1-2: Data presentation has been improved according to review comments in previous revised manuscript.

Changes in the text: There was no change in the text.

Comments 2: I'm also missing at least some basic information on genetics and on medical therapies (sirolimus is a good alternative to bleomycin for the treatment of microcystic LMs, this should at least be discussed).

Reply 2: The meaning of the reviewer was to add relevant discussion about genetics and medical therapies of other sclerosants, especially sirolimus. Until now, there was no genetic therapy on microcystic LMs. Medical therapies of other sclerosants have been discussed in Discussion. The reviewer emphasized sirolimus. Sirolimus is an immunosuppressor. It is always used in the treatment of complex LMs as its antiangiogenic and antiproliferative properties through oral administration. Our study mainly discussed sclerotherapy by injection, therefore, we did not add relevant comment in the Discussion section. In 2019, a clinical trial reported that 0.1% sirolimus cream was effective on treating cutaneous microcystic lymphatic malformations, providing a noval therapy, but sirolimus cream is not marketed and need further exploration.

Changes in the text: There was no change in the text.

Comments 3: Reference 9 does not state a very high complication rate for doxycycline.

Reply 3: The major and minor complication rates after doxycycline treatment were 2% and 10%, respectively. The dose of doxycycline injected per session ranged from 100 mg to 1000 mg with an average dose of 258 mg. However, its effect was positively related with the dosage. For young patients, dental staining should be taken into consideration after large volumes of drug.

Changes in the text: There was no change in the text.

Comments 4: Bleomycin also works when injected interstitially, this should be discussed. It is not necessary to inject it into the microcystic LMs themselves.

Reply 4: Bleomycin works when injected interstitially, we injected it into the microcystic LMs themselves because the lesions in our series were large and needed many times to treat, so it is much important to minimize its side effects. There would be more absorption into systemic circulation when injected interstitially and increases the risk of pulmonary fibrosis. In addition, sclerosants were always injected it into LMs themselves to perform a precise sclerotherapy.

Changes in the text: There was no change in the text.

Comments 5: Bleomycin does not primarily act by inducing inflammation, this is the mechanism of OK432, among other sclerosant. It seems to be more of a cytotoxic effect, which makes sense, since it a chemotherapeutic agent

Reply 5: In the studies about sclerotherapy of bleomycin, bleomycin was found to have a local sclerosing effect on endothelial cells of the cyst wall of LMs with nonspecific inflammatory reaction. It may be caused by cytotoxic effect. However, I'm sorry that I have not found the relevant indication in previous papers, so I didn't add it into my paper.

Changes in the text: There was no change in the text.

Comments 6: why use x-ray to detect lung fibrosis, lung function tests would be more appropriate.

Reply 6: Pulmonary function tests combined with radiographic assessment are more appropriate than either of them. We did not use pulmonary function tests because pulmonary function was always normal before severe pulmonary fibrosis, and chest X-rays was more available to observe pulmonary fibrosis. Currently, no standard follow-

up protocol is used in our hospital to quantify pulmonary function after use of bleomycin. We will add the examination in our further work.

Changes in the text: There was no change in the text.

Comments 7: precautions before using bleomycin should be mentioned (to avoid lung toxicity and hyperpigmentation)

Reply 7: The precautions were added into the text.

Changes in the text: (1) To minimize lung toxicity, intralesional bleomycin lavage was performed between the two scalp acupunctures at a concentration of 1mg/ml at a flow rate of 1ml/min in the larger cysts whose diameter was > 5mm detected by ultrasonic inspection and palpation (Figure 1). (2) The other complication that should be paid attention to was hyperpigmentation, laser treatment is effective to decrease it. In cases of superficial lesions of the face and neck with risk of subsequent poor cosmetic result, a dilution solution can be utilized.

Comments 8: Finally, there already many reports on the use of bleomycin in treating LM in the literature. Please could the authors comment, perhaps in the Discussion, what are the novel findings and/or how this article contribute to improve the readers' practice on treating LM.

Reply 8: We have added the clinical implication into the Discussion section.

Changes in the text: Until now, few published studies have evaluated the objective response in the treatment of bleomycin on large diffuse microcystic LMs. Our study, for the first time, demonstrated that the combined methods of intralesional lavage and intradermal injection were effective and safe to treat large diffuse microcystic LMs with low risk of complications and negligible lung toxicity. The success rate was 92.4% (excellent and moderate results), which was similar with the previous reports about bleomycin sclerotherapy in microcystic LMs.

Comments 9: Can the authors back up the statement on the safety and efficacy of bleomycin lavage with any data?

Reply 9: Sorry, we cannot provide quantitative data about safety and efficacy of bleomycin lavage. We used bleomycin lavage because several sessions were needed and the lesion was large, the drug dose should be controlled to diminish drug absorption. As you suggested, in our further work, the precise lavage volume should be calculated from intraluminal volume to improve our results. The limitation has been added into Discussion section.

Changes in the text: However, there was no quantitative data about lavage volume in each cyst, which will be calculated and documented in our further work to explore precise therapy.

Comments 10: The manuscript should be revised by a native speaker.

Reply 10: The manuscript was revised by a native speaker.