Peer Review File

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Reviewer A

Comment 1: The role of SAOS in AR treatment remains unclear to me; as far as I know, it is currently not suggested in treatment standards for AR. Clinical relevance of the results is missing.

Reply 1: SAOS is an emerging treatment option that focuses on modulating the immune system to address the underlying causes of allergic reactions. Derived from spleen extracts, SAOS are small bioactive molecules that can enhance the body's immune response. The solution is thought to work by:

(1) Immune Modulation: SAOS may help in regulating the immune system by promoting the balance of Th1 and Th2 cells, which are types of T-helper cells involved in allergic responses. This can reduce the hypersensitivity to allergens that triggers rhinitis symptoms.

(2) Anti-inflammatory Effects: SAOS might also exhibit anti-inflammatory properties, helping to reduce the inflammation in the nasal passages that contributes to the discomfort and congestion associated with allergic rhinitis.

(3) Enhancing Mucosal Immunity: By boosting the immune defenses of the mucous membranes, SAOS can potentially decrease the frequency and severity of allergic episodes.

The indications for SAOS include allergic disease like allergic rhinitis. A series of studies have confirmed its effectiveness, but most of the literature is in Chinese, with only a small portion available in English.

References:

[1] Feng, B., Zhang, W., Zheng, Q., et al. "Efficacy of Spleen Aminopeptide Oral Solution in the Treatment of Allergic Rhinitis and its Impact on T Lymphocyte Subgroup Immune Balance." *Chinese Journal of General Practice*, 2022, 19(12), 2038-2040, 2044. DOI:10.16766/j.cnki.issn.1674-4152.002230.

[2] Ma, T., Liu, S., Shi, H., et al. "Clinical Study on the Treatment of Seasonal Allergic Rhinitis with Spleen Aminopeptide Oral Solution." *Chinese Journal of Otorhinolaryngology Head and Neck Surgery*, 2022, 29(9), 596.

[3] Liu JT, Yang J, Guo R, Xi RQ, Wu YL, Chen C. Effect of Spleen Aminopeptide Oral Lyophilized Powder and Fluticasone/salmeterol Powder Inhaler on Pulmonary Function and Incidence of Adverse Reactions in Children with Cough Variant Asthma. Altern Ther Health Med. 2024 Jun 21:AT9521. Epub ahead of print. PMID: 38904627.

[4] Wu Y, Dong X, Wu R, Zheng X, Jin Y, Yang H. Efficacy and safety of spleen aminopeptide oral lyophilized powder in ameliorating liver injury in infants and children with human cytomegalovirus infection: a single-center study in China. Transl Pediatr. 2021 Jan;10(1):136-145. doi: 10.21037/tp-20-173. PMID: 33633945; PMCID: PMC7882289.

Changes in the text: In China, SAOS is widely used for the treatment of AR, hepatitis and other diseases, and it has been proven to be effective and safe. It is

an emerging treatment option that focuses on modulating the immune system to address the underlying causes of allergic reactions, including immune modulation, anti-inflammatory effects and mucosal immunity enhancement. (page 2, line 69-72)

Comment 2: In addition, a significant portion of participants was lost to follow-up. The authors have failed to describe whether and how this influenced results and interpretability of this study.

Reply 2: Since we use the PPS for efficacy evaluation, 22% of the children enrolled at baseline were excluded at the final data analysis. The smaller samples size may lead to false negative of the primary outcome as the type II error. We evaluated the power according to the results of the groups, as shown in the table below, the current study power is 95%. If we set the alpha=0.05, beta=0.1, study power quals to 90%, 32 cases for each group would meet the need for statistical analysis.

Control group N1(Mean, SD)	SAOS group N2(Mean, SD)	Alpha (Type I error)	Beta (Type II error)	Power (1-Beta)
36(0.70, 0.096)	42(0.62, 0.093)	0.05	0.051	0.949

References:

1.Rosner B. 2010. Fundamentals of Biostatistics. 7th Ed. Brooks/Cole.

2.Chow S, Shao J, Wang H. 2008. Sample Size Calculations in Clinical Research. 2nd Ed. Chapman & Hall/CRC Biostatistics Series.

Changes in the text: we added some discussion in the limitations (page 15, line 477-484)

Comment 3: Please make sure all abbreviations are written in full first time around, also in the abstract

Reply 3: Thank you for your advice, we have revised them.

Comment 4: Please evaluate correct use of English grammar throughout the whole article

Reply 4: Thank you very much for your advice, I have already found an editor whose native language is English to correct grammatical errors.

Comment 5: Line 60 and onwards: please refer to AR treatment guidelines and pose therapeutic options in order of these guidelines. Surgery is not the first choice treatment for rhintis.

Reply 5: The treatment for allergic rhinitis is relatively mature and relies on medication. However, there is currently no unified guideline for the treatment of adenoid hypertrophy, which primarily relies on medication, and surgical treatment is considered if drug therapy is ineffective. In the discussion section (page 14, line 384-393), I also introduced clinical treatment plans.

Line 384-393: Nasal steroids such as Beclomethasone [10, 19], Fluticasone [11, 12], Budesonide [13], and Mometasone [14] are prescribed for the treatment of pediatric AH. These medications have been shown to decrease the size of the

adenoids and alleviate symptoms of nasal obstruction [20]. Oral cysteine leukotriene receptor antagonists, such as Montelukast, are also used to manage AR during inflammatory episodes related to AH. Leukotriene receptor antagonists effectively reduce the A/N ratio and obstruction symptoms [21]. However, Montelukast has been associated with an increasing number of neuropsychiatric adverse drug reactions, particularly in children, including depression, sleep disturbance, and suicidal ideation [15]. Additionally, several adjunctive therapies are used to treat AH, including nasal irrigation, traditional Chinese medicine, bacteriotherapy, and halotherapy.

Comment 6: Line 65: please include references
Reply 6: we added the references.
References:
(1) Liu JT, Yang J, Guo R, Xi RQ, Wu YL, Chen C. Effect of Spleen Aminopeptide Oral Lyophilized Powder and Fluticasone/salmeterol Powder Inhaler on Pulmonary Function and Incidence of Adverse Reactions in Children with Cough Variant Asthma. Altern Ther Health Med. 2024 Jun 21:AT9521. Epub ahead of print. PMID: 38904627.
(2) Wu Y, Dong X, Wu R, Zheng X, Jin Y, Yang H. Efficacy and safety of spleen aminopeptide oral lyophilized powder in ameliorating liver injury in infants and children with human cytomegalovirus infection: a single-center study in China. Transl Pediatr. 2021 Jan;10(1):136-145. doi: 10.21037/tp-20-173. PMID: 33633945; PMCID: PMC7882289.

Comment 7: Figure 1: it is unclear how many people declined participation in this study
Reply 7: No children declined participate this study.

Comment 8: Line 143: please clarify if a visual analog scale was used
Reply 8: we have clarified a visual analog scale was used.
Changes in text: Nasal symptom score: A visual analog scale **(**10 cm long line**)** was used, with "no discomfort" (0) and "severe discomfort" (10) at the left and right ends, respectively. （page 4, line 141-142）

Comment 9: Line 147: by whom were the symptoms confirmed?
Reply 9: The family members marked the perceived level of discomfort on the line based on the clinical symptoms of the child (page 4, line 143-147)
Changes in the text: The family members marked the perceived level of discomfort on the line based on the clinical symptoms of the child (page 4, line 143-147)

Comment 10: Line 219: ... that met the inclusion...
Line 224: ... were presented...
Reply 10: we have corrected it to “who meet the inclusion”, however, we believe that the expression "were presented" is not problematic. First, "data" is plural, and

second, using the past tense is appropriate. If we have misunderstood your question, please let us know.
Changes in text: The per-protocol set (PPS), which includes all patients who meet the inclusion criteria, was used for the efficacy evaluation. (page 6, line 213-214)

Comment 11: Methods section: when (in/out AR Season) did the measurements take place?
Reply 11: The experiment did not specifically include patients during certain seasons. The patients we included are basically allergic to dust mites and pollen (page 3, line 94), with the majority being dust mite allergies, this type of allergy is perennial and not influenced by seasonal factors.

Comment 12: Table 2: why is the A/N ratio at T2 not reported?
Reply 12: A/N ratio is reported at T2 (8 weeks after treatment), but not reported at T1 (4 weeks after treatment). Because of the radiation in the X-rays, the family members are reluctant to take more X-rays.

Comment 13: Line 350: please clarify the protocol violations and why they were made
Reply 13: A total of 22 children were excluded, including 2 children who were excluded because their A/N ratio was 0.6 before medication, 3 children who voluntarily withdrew from the study, and 13 children who were lost to follow-up during medication monitoring. The primary reasons for voluntary withdrawal and loss to follow-up are likely due to the impact of COVID-19, with some children being quarantined at home or in other locations, making it impossible for them to attend follow-up visits on time. Moreover, the taste of SAOS is not good. However, despite these protocol violations, the final sample size in the PPS was still sufficient to assess the primary outcome, achieving 95% power.
Changes in the text: added some discussion (page 15, line 477-484).

Comment 14: Line 430: Please clarify whether the improvement in adenoid size was clinically relevant.
Reply 14: The improvement in adenoid size (A/N ratio) was clinically relevant. For the clinical symptoms of AH (nasal congestion, snoring, mouth breathing and restless sleep), after 8 weeks of treatment, the SAOS group showed more significant improvements compared to the control group. (Page 15, line 437-439)

Comment 15: Line 433: at what time?
Reply 15: we have revised the paragraph: After 8 weeks of treatment, the proportion of severe and moderate AH in the SAOS group (11.90%) was significantly lower than that in the control group (52.78%). （page 15, line 435）

Comment 16: Line 464: almost 20% of the study population was excluded due to protocol violations. How were results and study power influenced by this?

Reply 16: Since we use the PPS for efficacy evaluation, 22% of the children enrolled at baseline were excluded at the final data analysis. The smaller samples size may lead to false negative of the primary outcome as the type II error. We evaluated the power according to the results of the groups, as shown in the table below, the current study power is 95%. If we set the alpha=0.05, beta=0.1, study power quals to 90%, 32 cases for each group would meet the need for statistical analysis.

Control group N1(Mean, SD)	SAOS group N2(Mean, SD)	Alpha (Type I error)	Beta (Type II error)	Power (1-Beta)
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Reviewer B

Comment 1: Study Design: While the randomized controlled trial design is suitable for evaluating treatment efficacy, the absence of a placebo control raises concerns about potential bias, especially when considering subjective outcomes. A clear justification for omitting the placebo is essential.

Reply 1: This is an exploratory study, which offers a preliminary comparison with standard treatment methods. It initially investigates the feasibility of treating allergic rhinitis with adenoid hypertrophy using SAOS, providing a reference for future large-scale, multicenter, randomized, double-blind RCTs.

Comment 2: pleen Aminopeptide Oral Solution: Given the unfamiliar nature of SAOS as a Chinese Traditional Medicine, a more detailed explanation of its composition and mechanism of action is necessary. Although previous clinical studies are mentioned, comprehensive evidence on safety and efficacy, including dedicated Phase 1 and 2 trials assessing pharmacokinetics, pharmacodynamics, and safety, is crucial. Additional references supporting SAOS in lines 69-73 are recommended.

Reply 2: SAOS is not a traditional Chinese medicine; it is a biological preparation extracted from bovine spleens. It is not a single compound but contains thousands of nucleotides and small peptides. This complexity makes it challenging to conduct pharmacokinetics and pharmacodynamics studies. Although SAOS has been used clinically for over 40 years, there is still no Phase I or Phase II clinical data available in China.

A series of studies have confirmed its effectiveness, but most of the literature is in Chinese, with only a small portion available in English.

References:

[1] Feng, B., Zhang, W., Zheng, Q., et al. "Efficacy of Spleen Aminopeptide Oral Solution in the Treatment of Allergic Rhinitis and its Impact on T Lymphocyte Subgroup Immune Balance." *Chinese Journal of General Practice*, 2022, 19(12),

2038-2040, 2044. DOI:10.16766/j.cnki.issn.1674-4152.002230.
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[3] Liu JT, Yang J, Guo R, Xi RQ, Wu YL, Chen C. Effect of Spleen Aminopeptide Oral Lyophilized Powder and Fluticasone/salmeterol Powder Inhaler on Pulmonary Function and Incidence of Adverse Reactions in Children with Cough Variant Asthma. Altern Ther Health Med. 2024 Jun 21:AT9521. Epub ahead of print. PMID: 38904627.
[4] Wu Y, Dong X, Wu R, Zheng X, Jin Y, Yang H. Efficacy and safety of spleen aminopeptide oral lyophilized powder in ameliorating liver injury in infants and children with human cytomegalovirus infection: a single-center study in China. Transl Pediatr. 2021 Jan;10(1):136-145. doi: 10.21037/tp-20-173. PMID: 33633945; PMCID: PMC7882289.
Changes in the text: In China, SAOS is widely used for treating AR, hepatitis, and other diseases, and it has been proven to be effective and safe. It is an emerging treatment option that focuses on modulating the immune system to address the underlying causes of allergic reactions, including immune modulation, anti-inflammatory effects, and mucosal immunity enhancement [16,17]. (page 2, line 69-72)

Comment 3: Safety Evaluation: While CBC and urine exams were conducted, the absence of kidney and liver function assessments raises concerns about the adequacy of safety evaluation for a new medication. Further justification for omitting these tests is required.
Reply 3: Initially, the research protocol included liver and kidney function tests, but they required blood draws, which were too difficult for the children to cooperate with. Therefore, we decided to forgo them.

Comment 4: High Dropout Rate: The high dropout rate of 22% due to non-compliance demands further investigation. Detailed information on the reasons for non-compliance, such as taste, side effects, or other factors, is essential. A clear definition of non-compliance should be provided. While the study analyzed per-protocol data, an intention-to-treat analysis would strengthen the findings.
Reply 4: The primary reason for high drop rate was likely due to the impact of COVID-19, with some children being quarantined at home or in other locations, making it impossible for them to attend follow-up visits on time. Moreover, the taste of SAOS is unpleasant, and children do not like to drunk it.
Since we use the PPS for efficacy evaluation, 22% of the children enrolled at baseline were excluded at the final data analysis. The smaller samples size may lead to false negative of the primary outcome as the type II error. We evaluated the power according to the results of the groups, as shown in the table below, the current study power is 95%. If we set the alpha=0.05, beta=0.1, study power quals to 90%, 32 cases for each group would meet the need for statistical analysis.

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2.Chow S, Shao J, Wang H. 2008. Sample Size Calculations in Clinical Research. 2nd Ed. Chapman & Hall/CRC Biostatistics Series.

Comment 5: Table 5: The discrepancy between mean score differences (T0-T1 and T2-T0) being zero and significant Z-scores requires clarification and explanation.
Reply 5: Table 5 shows the comparison of differences in combined medication scores, with T0, T1, and T2 representing the combined medication scores before medication, 1 month after medication, and 2 months after medication, respectively, with higher scores indicating more combined medication.
T1-T0 means the reduction in combined use after 1 month of treatment minus before treatment.
T2-T0 means the reduction in combined use after 2 months of treatment minus before treatment.
Since the majority of the PPS population (55/78, 70.5%) did not have combined medication during the study period, the median (interquartile range, IQR) of the difference of combination medication score before and after treatment showed in the table were being zeros. The median difference test of (T0-T1 and T2-T0) showed that the difference in combined medication scores of (T0-T1 and T2-T0) was statistically significant.

Comment 6: Abstract: Please use the full names of allergic rhinitis (AR) and adenoid hypertrophy (AH) before their abbreviations.
Reply 6: we have revised it in the abstract.

Comment 7: Line 451-452: Replace the brand name "Claritan" with the generic name.
Reply 7: we have replaced the “Claritan” to “LoratadineSyrup”.

Comment 8: Discussion: The first paragraph of discussion should commence with a clear and concise summary of the study's primary findings to provide a strong foundation for subsequent analysis and interpretation.
Reply 8: thank you for your suggestions, we have added a paragraph in the discussion.
Changes in the text: A total of 100 children were enrolled in this study, with 22 children dropping out due to protocol violations. Based on the PPS dataset, 78 children were included, with 36 in the control group and 42 in the SAOS group. There were no significant differences in baseline A/N ratio, nasal symptom scores, or AH scores between the control and SAOS groups. However, after 8 weeks of treatment, no significant difference in nasal symptom scores between the two

groups. The A/N ratio in the SAOS group was significantly lower than in the control group, additionally, some AH scores (mouth breathing and restless sleep) showed significant improvement in the SAOS group. Moreover, SAOS reduced the need for additional medications in children, and no adverse events were observed during 8 weeks of treatment. (page 14, line 372-379)