Peer Review File

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Reviewer 1

Comment 1: The manuscript is well written and structured, although in my view too lengthy.
Reply 1: Thank you for taking the time to review and evaluate our manuscript. Trimming the text as guided by Comments 2-4 of this manuscript allows for a more focused review of this treatment in the setting of Hashimoto's thyroiditis.
Changes in Text: We have refined the manuscript using the below comments, reducing our word count by roughly 10% (4,723 words to 4,191 words; see Page 0, Line 21)

Comment 2: Where possible, the manuscript should be shortened where possible in particular regarding results of wet cupping in various disorders aside HT. Focus on the results available in HT.
Reply 2: Thank you for your suggestion. We have revised the manuscript where possible to concentrate on results specifically relating wet cupping to HT. In turn, this modification assists in making the manuscript more concise and focused. Because of the necessity of highlighting shared immunological and biological mechanisms imposed by the act of wet cupping, we have preserved examples of certain autoimmune conditions and other closely linked disorders found in the manuscript. The molecular-level pathways affected by this treatment must be informed by evidence-based, peer-reviewed sources, hence the need to draw upon related conditions to HT, which possess a more robust supply of literature to use as supporting evidence alongside articles that fit our search criteria and directly discuss application of this therapy in the context of HT.
Changes in Text: We have removed mention of wet cupping as it relates loosely to inflammatory disorders and analgesic effects as well as in-text discussion of thalassemia, functional diarrhea, and bronchial asthma (see Page 9, Line 230 & Page 12, Line 341).

Comment 3: Section 5, Page 13 can be deleted, as pain is not part of the clinical spectrum in HT.
Reply 3: Thank you for pointing out the lacking relevance of this section. We agree that this is outside of the clinical scope of Hashimoto's thyroiditis.
Changes in Text: We have removed *Section 5: Inflammation and Analgesic Effects* from the manuscript (see Page 12, Line 341).

Comment 4: Conclusion and future directions should be shortened. Too lengthy and repetitive.
Reply 4: We appreciate this feedback and share the opinion that the conclusion and future directions could be shortened. We find it particularly useful to remove the extent of explanation detailing potential clinical trials for future investigation due to exhibited redundancy.
Changes in Text: We have removed 3 of the paragraphs that repeat suggestions for future directions pertaining to the study design of possible clinical trials on p.15 of the manuscript (see Page 15, Line 462).

Reviewer 2

Comment 5: I think that the part about the autoimmune background of Hashimoto's thyroiditis could be improve. After a minor improvement through all or some of the aforementioned references, the paper could be published.
I suggest to you some useful references:

- Weetman AP. The immunopathogenesis of chronic autoimmune thyroiditis one century after hashimoto. Eur Thyroid J. 2013 Jan;1(4):243-50. doi: 10.1159/000343834. Epub 2012 Nov 2. PMID: 24783026; PMCID: PMC3821488.
- Mazzieri A, Montanucci P, Basta G, Calafiore R. The role behind the scenes of Tregs and Th17s in Hashimoto's thyroiditis: Toward a pivotal role of FOXP3 and BACH2. Front Immunol. 2022 Dec 12;13:1098243. doi: 10.3389/fimmu.2022.1098243. PMID: 36578493; PMCID: PMC9791026.
- Kristensen B, Hegedüs L, Madsen HO, Smith TJ, Nielsen CH. Altered balance between self-reactive T helper (Th)17 cells and Th10 cells and between full-length forkhead box protein 3 (FoxP3) and FoxP3 splice variants in Hashimoto's thyroiditis. Clin Exp Immunol. 2015 Apr;180(1):58-69. doi: 10.1111/cei.12557. PMID: 25412700; PMCID: PMC4367094.
- Tokić S, Štefanić M, Glavaš-Obrovac L, Jaman S, Novosadová E, Petrkova J, Navratilova Z, Suver Stević M, Petrek M. The Expression of T Cell FOXP3 and T-Bet Is Upregulated in Severe but Not Euthyroid Hashimoto's Thyroiditis. Mediators Inflamm. 2016;2016:3687420. doi: 10.1155/2016/3687420. Epub 2016 Jul 5. PMID: 27478306; PMCID: PMC4949338.
- Fichna M, Żurawek M, Słomiński B, Sumińska M, Czarnywojtek A, Rozwadowska N, Fichna P, Myśliwiec M, Ruchała M. Polymorphism in BACH2 gene is a marker of polyglandular autoimmunity. Endocrine. 2021 Oct;74(1):72-79. doi: 10.1007/s12020-021-02743-9. Epub 2021 May 8. PMID: 33966174; PMCID: PMC8440266.

Reply 5: Thank you for your interest in our manuscript and the provision of literature to help further strengthen our discussion on the role of autoimmunity in HT. Incorporating findings from these works into our manuscript assists in highlighting the different immunomodulatory components that make HT distinct. In the interest of subjecting further literature inclusion to the same search criteria implemented in our initial search methodology, we have carefully agreed upon 3 of the 5 listed references as eligible for inclusion. In this manuscript, we have outlined key players of the innate and adaptive immune system relevant to the benefits of cupping for Hashimoto’s thyroiditis to show that the pitfalls in pathological dysregulation among HT patients may be managed by the advantages of cupping practices. In favor of keeping within the scope of the review’s intended goals, we have strategically chosen to focus upon the specific elements of HT autoimmunity that align with the key mechanisms implicated in wet cupping practices.
Changes in Text: We have included reference to the resulting hallmarks of adaptive immunity in HT that were discovered by Kristensen et al. (2015) and Mazzieri et al. (2022) in the section *Immunomodulation of the Adaptive Immune System* (see Page 9-10, Lines 247-264), as well as referenced the article by Weetman (2013) in our section titled *Relating Immunological Therapies to Thyroid Function* (see Page 12, Lines 345-347).