Peer Review File

Article information: https://dx.doi.org/10.21037/abs-22-38

Reviewer A

The topic is important therefore the authors should be commended for their manuscript. It also with interest I learned a lot of the novel techniques and possibilities presented.

Comment 1: My concerns regarding the current MS are that it is very elaborate and not easy to read for clinicians. Who would be the authors preferred audience? As a comparison I read a review in Current Oncology on post-prostatectomy lymphedema by Bianchi et al 2023 which contained a lot of issues discussed in the current paper but for me as a clinician much easier to comprehend.

Reply 1: We agree that the MS might not be easiest for the clinician to read. However, we have done a great deal of basic research on the issue. As we were invited to write this review we assumed that this was something that wanted to be highlighted because behind clinically relevant innovations and clinical trials there are loads and loads of basic research. We should have discussed this in the beginning to have a more targeted audience. The revisions made are specified in the following answers.

Comment 2: I would highly recommend that the paper was more structured and less detailed as it includes so many experimental studies not yet published or with a design that it is hard for the reader to cap and draw conclusions from. I acknowledge that the definition of a narrative review is broad but generally aimed at identifying and summarizing what has previously been published including existing debates, previous studies on a certain topic, identifying knowledge gaps, and speculating on the latest interventions available.

Reply 2: We appreciate the comment. We have now revised the MS to be less detailed and more readable. The revisions are numerous and throughout the MS, so the revisions are not specified in this letter, however they are marked in red in the MS.

Comment 3: On p 23, exclusion criteria are mentioned papers with low reliability and in the submitted table. Table I find a lot of empty spaces and quote: not available, in progress or not printed. The study last referred to in table 1 CTX 4430, no data are available, should that study really be included in a narrative review? I question this and would ask the EIC and authors to reconsider this presentation. The title states Novel regenerative therapies, and very little is mentioned about diagnostics which might be of interest to the reader.

Reply 3: Thank you for your insightful comment. We have now revised the Table 1 and removed the study of CTX 4430, because of the lack of data. See the attached revised Table 1.

Comment4: I would have preferred a statement that there is no definitive cure for secondary lymphedema neither for upper nor for lower limb lymphedema.

Reply 4: Thank you for your remark. We have now added a sentence as follows to the MS.

Changes in the text:
Page 4, line 22: "*A definitive cure for secondary lymphedema is yet to be found*."

Comment 5:the MS is filled with abbreviations which really for a breast surgeon/plastic surgeon not in the immediate experimental field is unpleasant. Also, some abbreviations like Th2 cells are not written out and ADSC. Suggest making a glossary for all abbreviations in the beginning of the paper.

Reply 5: We agree that list of abbreviations would be helpful for the reader and hence we have now added it to the MS.

Changes in the text:
Page 3:
"***Abbreviations***
BCRL Breast cancer-related lymphedema
LN Lymph node
TGF-β1 Transforming growth factor beta 1
IL-10 Interleukin-10
Th2 Type 2 T-helper cell
TNF-α/-β Tumor necrosis factor alpha/beta
VEGF-C Vascular endothelial growth factor C
ALNT Autologous lymph node transfer
LVA Lymphatico-venous anastomosis
3D Three-dimensional
LEC Lymphatic endothelial cell
LIPO Liposuction
ADSC Adipose-derived stem cells
MRI Magnetic resonance imaging
LT- α Lymphotoxin-α (also known as TNF-β)
iPSC Induced pluripotent stem cell
LyQoLI Lymphedema Quality of Life Inventory
QOL Quality of life
HGF Hepatocyte growth factor
INF-γ Interferon gamma
IL-4 Interleukin 4
IL-13 Interleukin 13
NSAID Nonsteroidal anti-inflammatory drug
LTB_4 Leukotriene B_4
G-CSF Plasma granulocyte
LTA_4 Leukotriene A_4
VEGFR-3 Vascular endothelial growth factor receptor 3
SVF Stromal vascular fraction
TDC Tissue dielectric constant"

Comment 6: Further editorial comments that in my mind would make the paper easier to read would be a description of the methods more summarized and shortened as the results are so scarce and done on rat models.

Reply 6: We tried to now shorten description of methods. However, it is a relevant point whether a study is made in a rat (small animal) or porcine (large animal) model as the lymphatic system dynamics are different.

Comment 7: The first sentence of the manuscript could perhaps be omitted or rephrased in the context of this paper, as the second sentence explores early breast cancer treatment and the sentinel node concept which in well conduced RCTS (references to be included) have reduced morbidity in the axilla substantially. "Evacuation in the axillary area" is an unusual way of saying axillary lymph node clearance?

Reply 7: Thank you for your comment. We have now revised the first paragraph of the MS and added a reference.

Changes in the text:
Page 4, lines 4-10:" *…or LN evacuation (2). The iatrogenic damage to the lymph nodes and vessels and/or postoperative radiotherapy, chemotherapy, obesity and formation of seroma can cause a disruption of lymph flow causing lymphedema(3,4). The cumulative incidence of clinically manifested lymphedema after breast cancer treatment can be up to 41.1%(5,6). Chronic lymphedema first manifests with accumulation of interstitial fluid and pitting edema in the affected arm and is later accompanied with irreversible accumulation of fibro-adipose tissue and non-pitting edema(7)…*"

Comment 8: The figure submitted is clear and decorative and brightens up the paper but overtakes but sort of comes a little unexpected as no other figures of regeneration etc. are included.

Reply 8: The other studies are summarized in the Table 2, we thought of keeping the figure in the manuscript.

Comment 9: Looking through the extensive reference list and looking to the corresponding text I get confused, for instance ref 65 on p 13 is referred to in two sentences which seems odd.

Reply 9: Thank you for your meticulous insight. We have revised the references to be properly cited.

Comment 10: The quite extensive conclusion is quite comprehensive and maybe there should the sentence on non-existing therapy for lymphedema be more suited?

Reply 10: We appreciate the comment. We revised the conclusions to be more readable and removed excess information and explanations. Kindly see the answer 5, we have added the sentence of non-existing therapy in the introduction.

Changes in the text:

Page 15, lines 10-35 and page 16, lines 1-18:

"*Surgical procedures, such as ALNT and LVA, have yielded positive, but varying results in the treatment of lymphedema. Liposuction is an efficient debulking method at late stages of lymphedema. The experimental studies have combined these surgical treatments with the induction of lymphangiogenesis by lymphatic scaffolds or growth factors (BioBridge™, Lymfactin®). Both methods have obtained promising results in animal models. However, in the human studies BioBridge™ has not yet advanced to randomized prospective studies to prove efficacy. Lymfactin® was first investigated in a prospective Phase I trial with promising results on both volume reduction, lymphoscintigraphy and quality of life compared to baseline. Further, it was investigated in a randomized prospective Phase II trial and showed positive effects in favor of the Lymfactin® group only for the tissue dielectric constant (TDC) ratio, not the above-mentioned primary outcome measures. There have also been concerns regarding the potential of VEGF-C in promoting growth of possible dormant tumor cells or metastasis. VEGF-C is naturally expressed by the lymph nodes. Although VEGF-C expression is associated with a poor prognosis and increased metastasis risk in some cancer types, for breast cancer, the data is controversial and in some studies VEGF-C has even been associated with a better survival rate.(79–81) In the Lymfactin® trial, the drug was administered on the operating table, ex vivo to minimize distant effects.*

Studies targeting the inflammatory component of lymphedema (Th2 inhibition, LTB_4 inhibition) have also shown positive effects in experimental models. In clinical studies they have shown reduction in skin thickness, but not a reduction in the excess volume of the arm compared to baseline.

Therapies that include ADSCs are still scarce, but based on preclinical data, they are likely to affect in two ways: by promoting lymphangiogenesis and also by modulating inflammation through secretion of IL-10(82,83). ADSCs potentially can also modulate the fibrotic component of lymphedema, since they are used in hypertrophic scar treatment. However, the clinical studies are limited and the results less impressive than the preclinical studies.

*Although considerable progress in lymphedema research has been made, the clinical trials have not resulted in a major breakthrough. See supplementary material (**Table 2**). In experimental models, the defect in the lymphatic system and the following edema are very homogenous as opposed to patients. In the human patient, the pathophysiology of lymphedema after surgery and radiation includes fibrosis, inflammation, an extensive defect in the lymphatic system followed by lack of functionality in the lymphatics and at late stages, the accumulation of adipose tissue. The radiation induced scarring in the axilla is often extensive. As the problem is multifactorial, a multimodality approach seems to be the most feasible solution. A combination treatment with modulation of immune responses, induction of lymphangiogenesis combined with scar release and, also reconstructive surgery, would address several pathophysiology targets at once. ADSC treatment seems to also be a promising option. Clinical studies thus far are limited by the small number of patients and the comparison of studies is difficult because of the varying treatment, measurement, and follow-up protocols.*

There are promising novel therapies arising for BRCL. However, clinical studies are still scarce and experience from the above-mentioned clinical studies should be exploited to improve the quality, methodology and patient selection for future clinical trials.“

Reviewer B

I think this manuscript is interesting and valuable to be published. I suggested only a few points to revise.

Comment 1: Hepatocyte Growth Factor (HGF)
Some previous research suggested that HGF might improve lymphedema via promotion of lymphangiogenesis. Would you consider to add a new section about HGF? I suggested a reference below. If you think the section of HGF is not necessary, please mention the reason in the comments for me, which is not contained in the manuscript.
https://doi.org/10.1161/CIRCULATIONAHA.105.602953Circulation. 2006;114:1177–1184

Reply 1: We have now added a new chapter about hepatocyte growth factor. It was not included before as there are only limited publications available and the multiple not lymphangiogenesis related effects of HGF hinders its therapeutic benefits.

Changes in the text:

Page 10, lines 31-35 and page 11, lines 1-15:

"***Hepatocyte growth factor (HGF)***

Hepatocyte growth factor (HGF) has also been investigated regarding its role in lymphangiogenesis(50). HGF is now known to be able to regulate tissue and organ regeneration and modulate cell morphology. It can also stimulate cell motility and migration, and regulate cell growth and death.(51) HGF is highly interesting growth factor in the clinical setting due to the diverse potential in prognostic and therapeutic implications.

Kajiya et al.(51) found that LECs display elevated levels of HGF receptors compared to blood vascular cells. While the expression of HGF receptors is limited in normal lymphatic vessels, it increases in regenerating endothelium during tissue repair and in activated vessels during skin inflammation. Treating LECs with HGF boosts cell growth, migration, and tube formation. This highlights HGF's importance in lymphatic vessel growth and proposes HGF receptor as a potential target for managing irregular lymphatic growth.(51)

Saito et al.(52) examined the therapeutic potential of HGF in treating lymphedema. The researchers demonstrated that HGF treatment promoted cell growth, migration, and signaling pathways in LECs. Gene transfer of HGF in a rat model of lymphedema reduced swelling and increased expression of lymphatic markers. These findings highlight HGF's potential for enhancing lymphangiogenesis and treating lymphedema. However, no further studies on the exploitation of HGF have followed probably owing to the multiple other effects of HGF which makes it inspecific for lymphedema treatment.(52)"

Comment 2: TGF- β1

Authors commented about the involvement of TGF-β1 in the pathophysiology of lymphedema. I think the first report about the involvement of TGF-β1 is below (Cancer Sci. 2020;111(7):2620-2634. http://doi.org/10.1111/cas.14457). Please consider to add above to the references.

Reply 2: We have now added this reference.

Changes in the text:
Page 11, line 35: "…*situations(30,33,53)*."

Comment 3: VEGF-C
Authors mentioned VEGF-C therapy improved lymphedema. I also agree that. However, VEGF-C induce lymphangiogenesis and might promote lymph node metastasis. Therefore, administering of VEGF-C for the patients after cancer surgery should be performed very carefully. Authors should mention the potential to increase lymph node metastasis by VEGF-C treatment, and add the lymph node metastasis in the clinical studies.

Reply 3: VEGF-C does not promote tumor growth but rather lymphangiogenesis. In the clinical trials it has been injected ex vivo while the flap has been on the operating table. Also the expression is only transient lasting for only a weeks. VEGF-C is also naturally present in the lymph nodes (Saaristo et al Ann Surg 2012), of course at lower concentrations.

Changes in text: We have now discussed this better on page 15.
Comment 3: Table 1- Date of Search (specified to date, month and year)
According to our author instructions, please add "Date of Search (specified to date, month and year)" in the Table 1: https://abs.amegroups.org/pages/view/guidelines-for-authors#content-2-2-3