Peer Review File

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Reviewer A

Comment 1: Thank you for the opportunity to review. This paper certainly deals with a very interesting topic, which has become even more interesting also for complementary use after Covid-19 infection together with modern intensive care medicine. The paper is well written despite some quite lengthy sentences, e.g. page 3, lines 107-114, which hampers an easy flowing reading. Particularly this paragraph should be split up in two or three short sentences for better understanding.

Reply 1: Thank you for your useful comment. We have split up the long sentence cited above into 3 sentences and made changes taking into account the comments of Reviewer 2 (restriction to COVID-19 related topics only):

"Then, manual screening of the 16 words cited above referring to auricular vagus nerve neuromodulation was realized for the 44 articles. We ended up with only 16 articles (references 34-49)".

Changes in the text: We have modified our text as advised (see page 3, lines 111-113).

Comment 2: The paper has only one major problem. The concept and related format. As it probably was intended it touches a variety of potential applications of taVNS and tries to define the red line of its etiopathological background. For the very attractive purpose of a synopsis of existing evidence of the underlying pathway of action this paper does not reach far enough, although a number of relevant references have been included and cited.

For the format of a best-evidence paper with systematic review of the literature the search should focus on a better defined question and probably restricted to Covid-19 related topics only. As it is you have defined 12 papers dealing with different applications, but do not exclusively and extensively discuss these, but also refer to a number of papers not included in these best-evidence references. Please decide on one of these two alternatives, both will be interesting.

Reply 2: We acknowledge this major problem and we thank the reviewer for emphasizing it. We choose the second alternative that is to say, to focus on COVID-19 related topics and give an extensive discussion.

Changes in the text: As a consequence, we have realized major changes throughout the text: we have deleted the text dealing with endometriosis an cancer.

<u>In the former version</u> of the article, we have deleted

Page 1, Line 4 "underestimated nerve-driven diseases" replaced by "COVID-19 (line 3 of the new version)

Page 1, lines 21-22 "vagus nerve and endometriosis/vagus nerve and cancer progression"

Page 1, line 26 "nerve-driven diseases" replaced by "SARS-CoV-2 infection" (line

23 of the new version)
Page 2, Line 29-30 “nerve-driven diseases” replaced by “SARS-CoV-2 infection (line 27 of the new version)
Page 1, line 31-32 “endometriosis, cancer, epigenetics” replaced by “auricular branch of the vagus nerve, preventive treatment” (line 29-30 of the new version)
Page 2, Line 41-45 “Similarly, recent studies in oncology have demonstrated that nerves generate various neurosignaling pathways, which orchestrate cancer initiation, progression, and metastases (6). The best clinical evidence for the role of nerves in promoting tumorigenesis comes from prostate cancer patients where metastatic progression has been shown to be suppressed significantly in cases of spinal cord injury (7).”
Page 2, line 48 of the former version “notably with the current Coronavirus disease 2019 (COVID-19) pandemic” (line42-43 of the new version)
Page 2, line 68-69 “three examples of hot exoneural diseases, namely SARS-CoV-2 infection, endometriosis and cancers.” Replaced by “SARS-CoV-2 infection” (line 104 of the new version)
Page 3, lines 83-100 “Endometriosis, characterized by the presence of extra-uterine endometrium, is a common gynecological disorder in reproductive-age women. This estrogen-dependent disease features with chronic inflammation. Recent evidence demonstrates that the peripheral nervous system plays an important role in the pathophysiology of this disease. Both sensory and sympathetic peripheral nerves drive and maintain endometriotic changes. Sensory nerves, which surround and innervate endometriotic lesions, not only drive chronic pain but also contribute to a growth phenotype by secreting neurotrophic factors and interacting with surrounding immune cells (23, for a review). The diverse array of contributions that neurons play in endometriosis indicate that it should be considered as a nerve-driven disease (24-26).

Moreover, endometriosis has been recognized as a precursor lesion of several types of malignancies and endometriosis-associated carcinoma (27-30). Increasing evidence suggests that the nerves can also provide active inputs to tumors and there is two-way communication between nerves and cancer cells within the tumor microenvironment (31, for a review). Nerve inputs to tumors are derived mainly from the sympathetic (adrenergic) and the parasympathetic (cholinergic) systems, which are interactive. An important component of the latter is the vagus nerve, the largest of the cranial nerves. The balance of the sympathetic and parasympathetic contributions to early versus late tumorigenesis varies amongst the different cancers.”
Page 3 line 105 “/ vagus nerve and endometriosis/ vagus nerve and cancer and progression »
Page 3 line 106 “128 articles (respectively 61, 3 and 64 articles).” Replaced by “(page3 line 111, new version).
Page 4, line 125 “but by only one team for endometriosis (43) and by none at all for cancers.”

Page 4-5, lines 126-141 “The first explanation may reside in an inadequate

selection of the keywords used for cancer search: maybe it should have been more targeted on one precise type of cancer (contrary to the SARS-CoV-2 infection for instance, see also 44), or on the contrary, maybe the keyword "progression" (added to "cancer" in order to avoid symptomatic treatments) has narrowed the research inadvertently.

The second reason is that other types of vagus nerve stimulation seem to be preferred for cancer treatment (like invasive Vagus Nerve Stimulation, see 44) probably because cancer is a chronic disease and requires observance in the long-term.

At last, maybe the more recent a nerve-driven disease is acknowledged, the more likely auricular vagus nerve stimulation is selected among VNS techniques. Indeed, although the history of taVNS is short comparatively to invasive VNS, taVNS devices have been constantly updated and are likely to be considerably improved in the near future, presumably because it is an affordable and convenient technique (33).

As a consequence, it is very likely that auricular vagus nerve stimulation will gain importance in the therapeutic strategy of cancers within only a few years, provided its underlying mechanisms of action are better elucidated."

Page 5, lines 146-150 "including inflammatory conditions and cancers (49, for a review). It is noteworthy that patients suffering from endometriosis exhibit reduced vagal activity compared to controls, indicative of a disrupted autonomic balance (43) and that vagal tone assessed by HRV monitoring has been strongly correlated to cancer prognosis (50-52)."

Page 5, lines 152-153 "Epigenetics determine how the genotype of an organism responds to the environment in a coordinated way (53)."

Page 5, lines 162-163 "The development and progression of many pathological conditions (including infection, inflammatory and cancer) can be driven by aberrant epigenetic modifications."

Page 5, lines 166-172 "Regarding endometriosis (59) and cancer (60), there is emerging evidence implicating a crucial role for epigenetics. For instance, during tumorigenesis, the epigenome is subjected to various alterations such as global changes in histone modifications, dysregulation in the non-coding RNA networks, global loss of DNA methylation, and regional hypermethylation particularly in the promoter regions of tumor suppressor genes (61). Neuromodulation through ears could be a good alternative or complementary treatment to the so-called *epidrugs* (62-63), considering its harmlessness, especially in early stages of the diseases."

Page 6, lines 174-175 "by increasing its resilience, the latter being also linked to Heart Rate Variability (64-65)".

Page 6, lines 177-194 "Such clinical trials are all the more urgently needed that increased menstrual bleedings have been reported massively after receiving the anti-SARS-CoV-2 immunizing preparations (66-67).Given the fact that endometrial inflammasome is activated during menstruations (68) and the potentially increased tumorigenesis mediated by toll-like receptors signaling

pathways decrease reported in patients vaccinated against SARS-CoV-2 (69), noninvasive neuromodulation through ears seems to be the perfect complementary treatment in the COVID-19 pandemics, killing three birds with one stone." Thus, neuromodulation through ears could revolutionize the prevention of diseases, not only in pre-symptomatic SARS-CoV-2 infection (36), in early endometriosis, particularly with new diagnosis salivary test (70), but also in pre-cancerous stages (71**)** and in very early stages of life, as *in utero* period (72). This unique feature has been too little examined in the context of neuromodulation in pediatrics. By taking advantage of crucial and critical and sensitive periods of neurodevelopment that control and shape the neuroplasticity potential of the developing brain (73), neuromodulation through ears might open "windows of opportunity" not seen in adults, although transcutaneous electrical stimulation of the spinal networks has already been able to restore movement and function of the limbs in patients with both complete paralysis and long-term spinal cord injury (74)."
Page 6, line 198 "at least SARS-CoV-2 infection, endometriosis and cancers".
Page 6, lines 199-201 "of infectious, immune-mediated as well as in neoplastic conditions, on the basis of the regulation of the host's both homeostasis and resilience".
MOREOVER, text was added: pages and lines are indicated in the new version below:
Page 2, line 55-57: "Unexpectedly, a few authors have suggested that therapeutic solutions to COVID-19 pandemic might even come from neuroscience providing a right understanding of the physiopathological mechanism of infection at CNS (14-16)."
Page 3, lines 98-100 "Unexpectedly, the emerging consensus arising that COVID-19 outcome depends on neurological issues, may suddenly boost the field of auricular vagus nerve neuromodulation". It is remarkable that Heart Rate Variability…"

Almost the whole discussion was modified…
Page3, lines 108-111 "This narrative literature review consisted of a MEDLINE search of English language publications between January 2000 to February 2022. The keywords used for the search were consecutively: vagus nerve and COVID-19 (MeSH Terms) OR vagus nerve and COVID-19 (other terms). This first step released 44 articles."

Page4-5, lines 116-187: "Our non-exhaustive review (limited to Medline, including only written articles in English and using few keywords) confirms that auricular vagus nerve neuromodulation has been suggested by several scientific teams as a therapeutic solution for the COVID-19 pandemic. In the 16 articles retrieved (34-49), taVNS is the mostly used method (only one article deals with the use of semi-permanent needles, 38) to target respiratory symptoms or mental health_

Our search has retrieved 7 reviews (34, 36, 37, 43, 44, 46, 47), 6 Medical Hypotheses (35, 39, 40, 41, 42, 48), 1 case-report on 2 patients (45), and 2 randomized controlled studies (1 preclinical study (49) and only 1 human clinical trial, (38)). This probably reflects the difficulty to launch well-designed clinical trial dealing with non-pharmacological treatments during the pandemic. Indeed, universities and hospital research centers who received major grants for COVID-19 research were mainly interested in pharmacological solutions, traditionally used in the field of immunology and infectious diseases. It is remarkable that the emergency use authorization (EUA) for the treatment of COVID-19 associated dyspnea obtained by the "gammaCore Sapphire™ CV", a cervical VNS, another type of noninvasive VNS distinct of auricular VNS, was not supported by a significant result from a randomized controlled clinical trial (http://www.fda.gov/media/139968/download; accessed July 19, 2021). Indeed, the results of the SAVIOR study have not been published yet (51).

The reviews and Medical hypothese articles found advocate for auricular vagus nerve neuromodulation therapeutic use during the current pandemic but only cite this option among others, without proving its efficiency. The case-report (45) relates the positive clinical outcome of 2 SARS-CoV-2-infected men (60 and 64 years-old respectively) treated by taVNS on both ears (4-5 mA current intensity,133-kHz decrescendo alternating current in bursts of 0,3 ms, 25Hz, during 60 minutes per day, once in the morning), starting roughly one week after the beginning of the COVID-19 symptoms until recovery (after 8 and 11 days of taVNS treatment respectively), in addition to usual drug treatment (including azithromycin and intravenous injection of methylprednisolone). However, the unmentioned comorbidities of the patients (therefore their prognosis factor), the use of validated efficient drug treatment (injections of methylprednisolone) and the lack of controls cannot allow to distinguish between a spontaneous positive outcome and a potential therapeutic efficiency of taVNS. Nevertheless, this case report proves the feasibility and the good tolerance of taVNS in stage 3 COVID-19 patients.

Moreover, the very recent Korean preclinical randomized controlled study (49), clearly demonstrates the anti-inflammatory effect of taVNS (200 µA, pulse width 200µs, pulse frequency 15 or 25 Hz, duration of stimulation: 5 or 10 minutes; n= 5 to 10 animals) in lipopolysaccharide - treated C57BL/6 mice, via activation of the Cholinergic Anti-Inflammatory Pathway (CAP) (5, 52). taVNS significantly decreased pro-inflammatory cytokines blood levels as well as CAP-target tissue inflammation (spleen, lung and intestine), depending on the applied frequency (the lower 15 Hz frequency being systematically significantly more efficient). Therefore, electrical auricular vagus nerve neuromodulation was shown to efficiently inhibit an acute systemic and tissue inflammation (but not literally resulting from SARS-CoV-2 infection) through activation of CAP in an animal model, provided the right frequency selection.

Unfortunately, the only clinical trials on auricular vagus nerve neuromodulation and COVID-19 (38), although randomized, controlled and

double-blind, was not able to show any significant effect of auricular vagus nerve. This is probably due to the small size of the population samples in each arm (14 in the auricular neuromodulation group and 15 in the sham group). Indeed, the study was stopped prematurely after a mid-term evaluation requested by the Ethical committee. Inclusions had not been pondered according to prognosis factors. Thus, by that time, the two arms of the study were not comparable (the auricular neuromodulation group having a median age ten years older and more masculine (80 % of men versus <50% in the sham group). Therefore, the absence of significance reported by this unique clinical trial does not conclude that auricular vagus nerve neuromodulation is ineffective.

Likewise, other clinical observations, published poorly understood so far, strongly support the idea that VNS is able to improve the outcome of COVID-19 patients. For instance, knowing that the spleen is the CAP main target organ, it is noteworthy that splenectomized patients have a higher risk of hospitalization or death during SARS-CoV-2 infection (RR 1.44; 53). Moreover, recent vagotomy experiments have proven that vagus nerve is essential to Hypothalamic Pituitary Axis (HPA) (54), probably via the secretion of Prolactin Releasing Peptide from NTS neurons to hypothalamic neurons (26). As a result, the reactive physiological activation of vagus nerve during viral infections, including SARS-CoV-2 infection, could be responsible for the observed hyperprolactinemia (55). Conversely, this could explain why HIV patients with hypoprolactinemia had higher risk of death from opportunistic infections (56). Last but not least, the paradoxical "Happy Hypoxemia" also argues for a crucial central role of vagus nerve during SARS-CoV-2 infection. Indeed, as mentioned earlier (26), NTS neurons are involved in interoceptive signals transmission from the body to the brain. SARS-CoV-2 invasion of NTS after a retrograde trafficking from the lung, via the vagus nerve, might be responsible for turning "Happy Hypoxemia" on. Auricular vagus nerve neuromodulation does not seem to be able to prevent SARS-CoV-2 infection (53, 57) but is more likely to lower the host vulnerability to the virus, probably through epigenetics modulations."

Page 5, lines 193-204 "Therefore, auricular vagus nerve neuromodulation would be indicated, as a priority, to the elderly men with co-morbidities (overweight, diabetes, hypertension…). The latter target population happens to be the one with the lowest measure of HRV (63), and as auricular vagus nerve neuromodulation has been shown to increase HRV (64), this rationale fits perfectly.

In addition to be safe and suitable for the vulnerable target population, auricular vagus nerve neuromodulation is easy to learn by non-medical staff (65) and can be affordable worldwide, depending on the type of stimulation (the treatment with semipermanent needles described in reference 38 costs less than 3 euros). At last, boosting the host's defenses against pathogens, whatever their identity, (notably the emerging virulent SARS CoV-2 variants) makes auricular

vagus nerve neuromodulation the ideal adjuvant therapeutic tool for pandemics, for both curative and preventive purposes."
The conclusion was modified:
Page 5, lines 207-211 "Although no validated recommendations for clinicians could be drawn from this narrative review, the converging arguments conveyed advocate auricular vagus nerve neuromodulation as an adjuvant therapeutic option to fully consider in pandemics. Larger studies are definitely needed to optimize the stimulation parameters, requiring a stronger support from governments and public research concern."

References were modified:
References deleted: (number of the reference, pages and lines are given from the former version):

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Reviewer B

This is an interesting, nicely written and appropriately referenced manuscript. Neuromodulation through ears is an underestimated therapeutic approach and the authors have done a good job in describing its value and exploring the evidence so far in treating human diseases. The following points should be taken into consideration.

Comment 1: Auricular vagus nerve stimulation. The manuscript should incorporate more description of the protocoles used to achieve auricular vagus nerve stimulation. A dedicated paragraph (instead of 1-2 sentences) would help the non-specialised reader to better understand what it is and how it is

practically done.

Reply 1: We thank the reviewer for this helpful comment. We introduced then a 3 dedicated paragraph presenting auricular vagus nerve stimulation as follows:

"Ear neuromodulation is particularly interesting because it can remotely target the otherwise hardly accessible brain (22-24), allowing non-invasive targeting of the brainstem (23-25). Indeed the human ear is the only superficial organ, thus easily accessible, supplied by the Vagus Nerve (Auricular Branch of the Vagus Nerve, ABVN). Then, the afferent fibers of the ABVN project to the Nucleus of the Solitary Tract (NTS), a critical central node relaying interoceptive feedback from body to the brain (for a review, 26). Applying the current to the skin of dedicated areas of the ear, and not necessarily directly to the vagus nerve, allows a rapid translation into clinical trials of the preclinical invasive Vagus Nerve Stimulation results. However, the rise of auricular vagus nerve neuromodulation has been slowed down by its lack of standardization.

First, its nomenclature is definitely heterogeneous whatever the nature of the ear stimulation, either electrical or physical. Electrical auricular Vagus Nerve Stimulation (VNS) gathers by itself 10 different terms (27-28). It is designed as auricular VNS (aVNS or AVNS), auricular transcutaneous vagus stimulation (atVNS), low-level tragus nerve stimulation (LL-TNS), low-level tragus electrical stimulation (LLTS), percutaneous auricular VN stimulation (PVNS), respiratory-gated auricular vagal afferent nerve stimulation (RAVANS), Motor Activated Auricular Vagus Nerve Stimulation (MAAVNS), transcutaneous auricular VN stimulation (taVNS or ta-VNS), transcutaneous tragus nerve stimulation (TNS), and transcutaneous VN stimulation (tVNS or TVNS). taVNS and tVNS are the most frequently used terminologies. On the other hand, physical neuromodulation through ears includes at least 6 terms: ear acupressure, auricular acupressure, ear acupuncture, auricular acupuncture, auriculotherapy or Auricular Neuromodulation. Therefore, we chose the periphrasis "Auricular Vagus Nerve Neuromodulation" to represent all the different terminologies cited above.

To further complicate the issue, the stimulation parameters and the target areas of the ear may differ widely among research teams. Many parameters, except current intensity and frequency, vary without standardized pattern. In the field of human neurological trials, frequency is usually set between 20 and 30 Hz and intensity, between perceptual and pain threshold (range 0.1-10 mA) (for a review, 29). Fortunately, closed-loop systems (RAVANS, MAAVNS) are about to solve this parametric problem (29)."

Changes in the text: We have modified our text as wisely advised (see pages 2-3, lines 68-97, in the new version of the manuscript).

Comment 2: In the introduction, "The best clinical evidence for the role of nerves in promoting tumorigenesis comes from prostate cancer patients where metastatic progression has been shown to be suppressed significantly in cases of spinal cord injury (7)". The following reference should also be added: Rutledge et

al Trends in Cancer 2017, PMID: 29198437.
Reply 2: We thank the reviewer for this comment. Nevertheless, since we restrained our review to COVID-19 (following the advice of Reviewer A), the sentence and its reference have been suppressed.
Changes in the text: We have modified our text as advised (see page 2, line 40).